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FIG.1A. NUCLEOTIDE SEQUENCE OF THE PIV-3 F GENE (PCR-AMPLIFIED)

```
AAGTCAATACCAACAACTATTAGCAGTCATACGTGCAAGAACAAGAAAGAAGAGATTCAA
TTCAGTTATGGTTGTTGATAATCGTCAGTATGCACGTTCTTGTTCTTTCTTCTCTAAGTT
          10        20        30        40        50        60

AAAGCTAAATAAGAGAAATCAAAACAAAAGGTATAGAACACCCGAACAACAAAATCAAAA
TTTCGATTTATTCTCTTTAGTTTTGTTTTCCATATCTTGTGGGCTTGTTGTTTTAGTTTT
          70        80        90        100       110       120

CATCCAATCCATTTTAAACAAAAATTCCAAAAGAGACCGGCAACACAACAAGCACCAAAC
GTAGGTTAGGTAAAATTTGTTTTTAAGGTTTTCTCTGGCCGTTGTGTTGTTCGTGGTTTG
          130       140       150       160       170       180
          <-------- SP --------
     MET PRO THR [LEU] ILE LEU LEU ILE ILE THR THR MET ILE MET ALA [SER] SER CYS GLN
ACAATGCCAACTTTAATACTGCTAATTATTACAACAATGATTATGGCATCTTCCTGCCAA
TGTTACGGTTGAAATTATGACGATTAATAATGTTGTTACTAATACCGTAGAAGGACGGTT
          190       200       210       220       230       240

 ILE ASP ILE THR LYS LEU GLN HIS VAL GLY VAL LEU VAL ASN SER PRO LYS GLY MET LYS
ATACATATCACAAAACTACAGCATGTAGGTGTATTGGTCAACAGTCCCAAAGGGATGAAG
TATGTATAGTGTTTTGATGTCGTACATCCACATAACCAGTTGTCAGGGTTTCCCTACTTC
          250       260       270       280       290       300

 ILE SER GLN ASN PHE GLU THR ARG TYR LEU ILE LEU SER LEU ILE PRO LYS ILE GLU ASP
ATATCACAAAACTTCGAAACAAGATATCTAATTTTGAGCCTCATACCAAAAATAGAAGAC
TATAGTGTTTTGAAGCTTTGTTCTATAGATTAAAACTCGGAGTATGGTTTTTATCTTCTG
          310       320       330       340       350       360

 SER ASN SER CYS GLY ASP GLN GLN ILE LYS GLN TYR LYS ARG LEU LEU ASP ARG LEU ILE
TCTAACTCTTGTGGTGACCAACAGATCAAACAATACAAGAGGTTATTGGATAGACTGATC
AGATTGAGAACACCACTGGTTGTCTAGTTTGTTATGTTCTCCAATAACCTATCTGACTAG
          370       380       390       400       410       420
```

FIG.1B.

```
ILE PRO LEU TYR ASP GLY LEU ARG LEU GLN LYS ASP VAL ILE VAL [THR] ASN GLN GLU SER
ATCCCTCTATATGATGGATTAAGATTACAGAAAGATGTGATAGTAACCAATCAAGAATCC
TAGGGAGATATACTACCTAATTCTAATGTCTTTCTACACTATCATTGGTTAGTTCTTAGG
         430        440        450        460        470        480

                                F2-F1 CLEAVAGE SITE
ASN GLU ASN THR ASP PRO ARG THR [ARG] ARG↓[SER] PHE GLY GLY VAL ILE GLY THR ILE ALA
AATGAAAACACTGATCCCAGAACAAGACGATCCTTTGGAGGGGTAATTGGAACCATTGCT
TTACTTTTGTGACTAGGGTCTTGTTCTGCTAGGAAACCTCCCCATTAACCTTGGTAACGA
         490        500        510        520        530        540

LEU GLY VAL ALA THR SER ALA GLN ILE THR ALA ALA VAL ALA LEU VAL GLU ALA LYS GLN
CTGGGAGTAGCAACCTCAGCACAAATTACAGCGGCAGTTGCTCTGGTTGAAGCCAAGCAG
GACCCTCATCGTTGGAGTCGTGTTTAATGTCGCCGTCAACGAGACCAACTTCGGTTCGTC
         550        560        570        580        590        600

ALA [LYS] SER ASP ILE GLU LYS LEU LYS GLU ALA ILE ARG ASP THR ASN LYS ALA VAL GLN
GCAAAATCACACATCGAAAAACTCAAAGAAGCAATCAGGGACACAAACAAAGCAGTGCAG
CGTTTTAGTGTGTAGCTTTTTGAGTTTCTTCGTTAGTCCCTGTGTTTGTTTCGTCACGTC
         610        620        630        640        650        660

SER VAL GLN SER SER ILE GLY ASN LEU ILE VAL ALA ILE LYS SER VAL GLN ASP TYR VAL
TCAGTTCAGAGCTCTATAGGAAATTTAATAGTAGCAATTAAATCAGTCCAAGATTATGTC
AGTCAAGTCTCGAGATATCCTTTAAATTATCATCGTTAATTTAGTCAGGTTCTAATACAG
         670        680        690        700        710        720

ASN [ASN] GLU ILE VAL PRO SER ILE ALA ARG LEU GLY CYS GLU ALA ALA GLY LEU GLN LEU
AACAACGAAATCGTGCCATCGATTGCTAGACTAGGTTGTGAAGCAGCAGGACTTCAATTA
TTGTTGCTTTAGCACGGTAGCTAACGATCTGATCCAACACTTCGTCGTCCTGAAGTTAAT
         730        740        750        760        770        780

GLY ILE ALA LEU THR GLN HIS TYR SER GLU LEU THR ASN ILE PHE GLY ASP ASN ILE GLY
GGAATTGCATTAACACAGCATTACTCAGAATTAACAAACATATTTGGTGATAACATAGGA
CCTTAACGTAATTGTGTCGTAATGAGTCTTAATTGTTTGTATAAACCACTATTGTATCCT
         790        800        810        820        830        840
```

FIG.1C.

```
SER LEU GLN GLU LYS GLY ILE LYS LEU GLN GLY ILE ALA SER LEU TYR ARG THR ASN ILE
TCGTTACAAGAAAAAGGAATAAAATTACAAGGTATAGCATCATTATACCGCACAAATATC
AGCAATGTTCTTTTTCCTTATTTTAATGTTCCATATCGTAGTAATATGGCGTGTTTATAG
         850        860        870        880        890        900

THR GLU ILE PHE THR THR SER THR VAL ASP LYS TYR ASP ILE TYR ASP LEU LEU PHE THR
ACAGAAATATTCACAACATCAACAGTTGATAAATATGATATCTATGATCTATTATTTACA
TGTCTTTATAAGTGTTGTAGTTGTCAACTATTTATACTATAGATACTAGATAATAAATGT
         910        920        930        940        950        960

GLU SER ILE LYS VAL ARG VAL ILE ASP VAL ASP LEU ASN ASP TYR SER ILE THR LEU GLN
GAATCAATAAAGGTGAGAGTTATAGATGTTGATTTGAATGATTACTCAATCACCCTCCAA
CTTAGTTATTTCCACTCTCAATATCTACAACTAAACTTACTAATGAGTTAGTGGGAGGTT
         970        980        990       1000       1010       1020

VAL ARG LEU PRO LEU LEU THR ARG LEU LEU ASN THR GLN ILE TYR [LYS] VAL ASP SER ILE
GTCAGACTCCCTTTATTAACTAGGCTGCTGAACACTCAGATCTACAAAGTAGATTCCATA
CAGTCTGAGGGAAATAATTGATCCGACGACTTGTGAGTCTAGATGTTTCATCTAAGGTAT
        1030       1040       1050       1060       1070       1080

SER TYR ASN ILE GLN ASN ARG GLU TRP TYR ILE PRO LEU PRO SER HIS ILE MET THR LYS
TCATATAATATCCAAAACAGAGAATGGTATATCCCTCTTCCCAGCCATATCATGACGAAA
AGTATATTATAGGTTTTGTCTCTTACCATATAGGGAGAAGGGTCGGTATAGTACTGCTTT
        1090       1100       1110       1120       1130       1140

GLY ALA PKE LEU GLY GLY ALA ASP VAL LYS GLU CYS ILE GLU ALA PHE SER SER TYR ILE
GGGGCATTTCTAGGTGGAGCAGATGTCAAGGAATGTATAGAAGCATTCAGCAGTTATATA
CCCCGTAAAGATCCACCTCGTCTACAGTTCCTTACATATCTTCGTAAGTCGTCAATATAT
        1150       1160       1170       1180       1190       1200

CYS PRO SER ASP PRO GLY PHE VAL LEU ASN HIS GLU KET GLU SER CYS LEU SER GLY ASN
TGCCCTTCTGATCCAGGATTTGTACTAAACCATGAAATGGAGAGCTGCTTATCAGGAAAC
ACGGGAAGACTAGGTCCTAAACATGATTTGGTACTTTACCTCTCGACGAATAGTCCTTTG
        1210       1220       1230       1240       1250       1260
```

FIG.1D.

ILE SER GLN CYS PRO ARG THR [THR] VAL [THR] SER ASP ILE VAL PRO ARG TYR ALA PHE VAL
ATATCCCAATGTCCAAGAACCACGGTCACATCAGACATTGTTCCAAGATATGCATTCGTC
TATAGGGTTACAGGTTCTTGGTGCCAGTGTAGTCTGTAACAAGGTTCTATACGTAAGCAG
1270 1280 1290 1300 1310 1320

ASN GLY GLY VAL VAL ALA ASN CYS ILE THR THR THR CYS THR CYS ASN GLY ILE [ASP] ASN
AATGGAGGAGTGGTTGCAAACTGTATAACAACCACCTGTACATGCAACGGAATCGACAAT
TTACCTCCTCACCAACGTTTGACATATTGTTGGTGGACATGTACGTTGCCTTAGCTGTTA
1330 1340 1350 1360 1370 1380

ARG ILE ASN GLN PRO PRO ASP GLN GLY VAL LYS ILE ILE THR HIS LYS GLU CYS ASN THR
AGAATCAATCAACCACCTGATCAAGGAGTAAAAATTATAACACATAAAGAATGTAATACA
TCTTAGTTAGTTGGTGGACTAGTTCCTCATTTTTAATATTGTGTATTTCTTACATTATGT
1390 1400 1410 1420 1430 1440

ILE GLY ILE ASN GLY MET LEU PHE ASN THR ASN LYS GLU GLY THR LEU ALA PHE TYR THR
ATAGGTATCAACGGAATGCTGTTCAATACAAATAAAGAAGGAACTCTTGCATTCTACACA
TATCCATAGTTGCCTTACGACAAGTTATGTTTATTTCTTCCTTGAGAACGTAAGATGTGT
1450 1460 1470 1480 1490 1500

PRO ASN ASP ILE THR LEU ASN ASN SER VAL ALA LEU ASP PRO ILE ASP ILE SER ILE GLU
CCAAATGATATAACACTAAATAATTCTGTTGCACTTGATCCAATTGACATATCAATCGAG
GGTTTACTATATTGTGATTTATTAAGACAACGTGAACTAGGTTAACTGTATAGTTAGCTC
1510 1520 1530 1540 1550 1560

LEU ASN LYS ALA LYS SER ASP LEU GLU GLU SER LYS GLU TRP ILE ARG ARG SER ASN GLN
CTTAACAAAGCCAAATCAGATCTAGAAGAATCAAAAGAATGGATAAGAAGGTCAAATCAA
GAATTGTTTCGGTTTAGTCTAGATCTTCTTAGTTTTCTTACCTATTCTTCCAGTTTAGTT
1570 1580 1590 1600 1610 1620

TM

LYS LEU ASP SER ILE GLY ASN TRP HIS GLN SER SER THR THR ILE ILE ILE [ILE] LEU ILE
AAACTAGATTCTATTGGAAACTGGCATCAATCTAGCACTACAATCATAATTATTTTAATA
TTTGATCTAAGATAACCTTTGACCGTAGTTAGATCGTGATGTTAGTATTAATAAAATTAT
1630 1640 1650 1660 1670 1680

FIG.1E

MET ILE ILE ILE LEU PHE ILE ILE ASN VAL THR ILE ILE THR ILE ALA ILE LYS TYR TYR
ATGATCATTATATTGTTTATAATTAATGTAACGATAATTACAATTGCAATTAAGTATTAC
TACTAGTAATATAACAAATATTAATTACATTGCTATTAATGTTAACGTTAATTCATAATG
1690 1700 1710 1720 1730 1740

ARG ILE GLN LYS ARG ASN ARG VAL ASP GLN ASN ASP LYS PRO TYR VAL LEU THR ASN LYS
AGAATTCAAAAGAGAAATCGAGTGGATCAAAATGACAAGCCATATGTACTAACAAACAAA
TCTTAAGTTTTCTCTTTAGCTCACCTAGTTTTACTGTTCGGTATACATGATTGTTTGTTT
1750 1760 1770 1780 1790 1800

TGACATATCTATAGATCATTAGATATTAAAATTATAAAAAACTT
ACTGTATAGATATCTAGTAATCTATAATTTTAATATTTTTTGAA
1810 1820 1830 1840

NUCLEOTIDE SEQUENCE OF THE PIV-3 F GENE. THE cDNA SEQUENCE IS SHOWN IN THE PLUS (mRNA) STRAND SENSE IN THE 5' TO 3' DIRECTION. THE SIGNAL PEPTIDE (SP) AND THE TRANSMEMBRANE (TM) ANCHOR DOMAIN ARE UNDERLINED. THE PREDICTED F2-F1 CLEAVAGE SITE IS INDICATED BY THE ARROW (↓). AMINO ACIDS DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY THE PIV-3 F GENE ARE BOXED.

RESTRICTION MAP OF THE PIV-3 F GENE

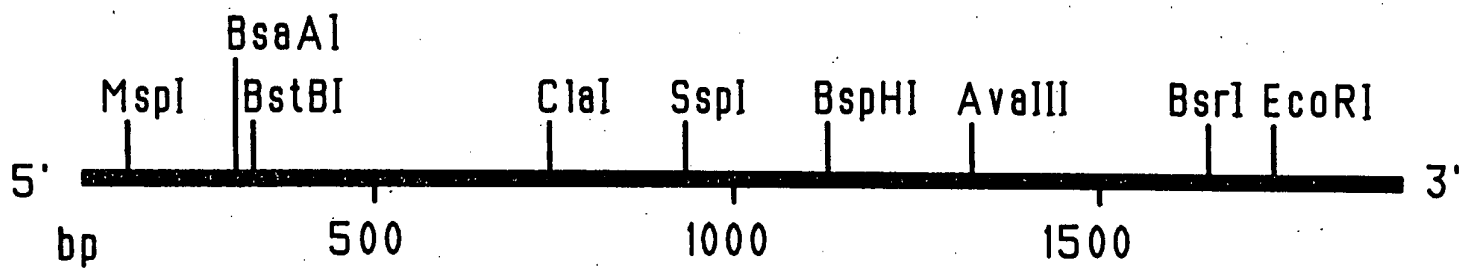
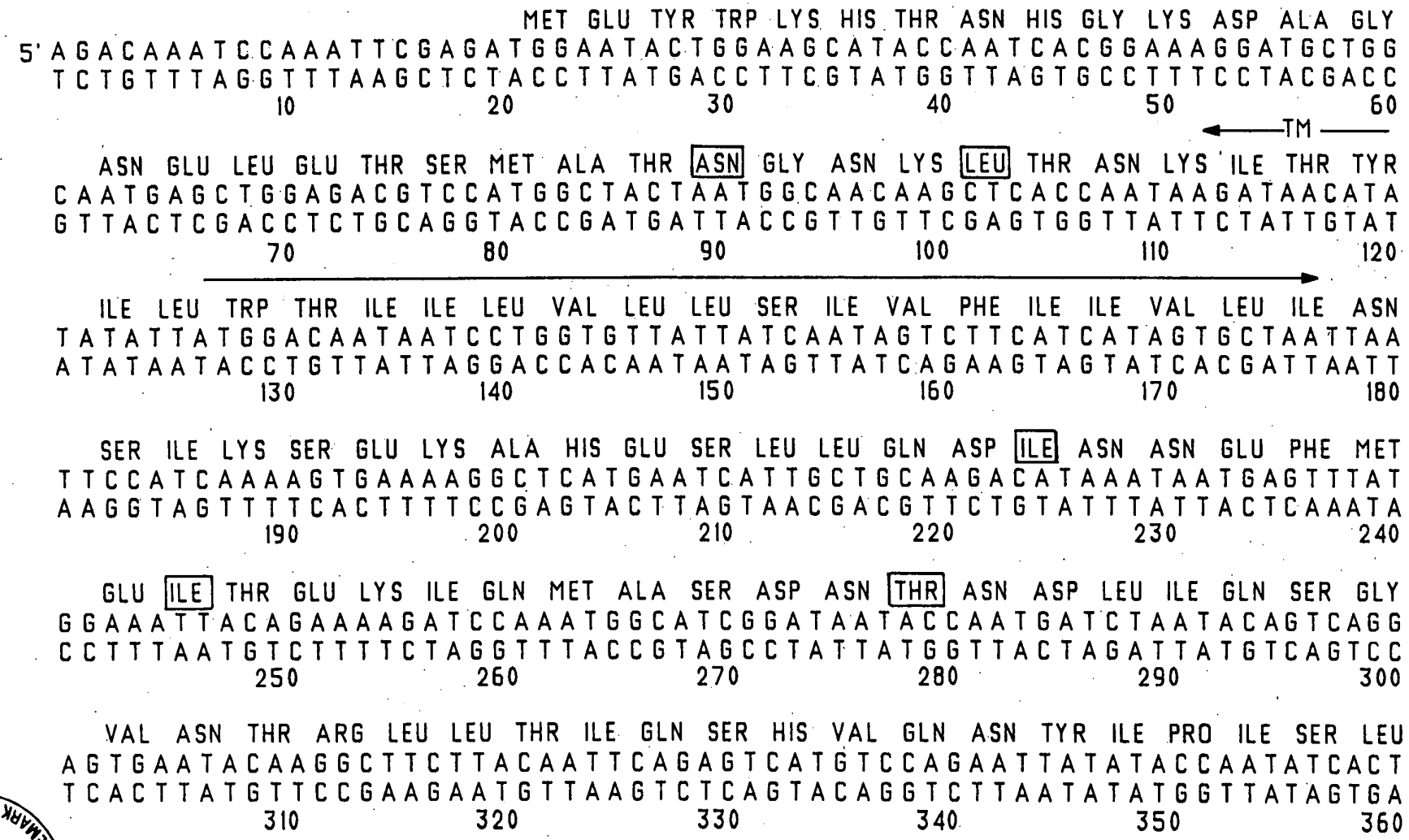


FIG.2.

FIG.3A. NUCLEOTIDE SEQUENCE OF THE PIV-3 HN GENE.



THR GLN GLN MET SER ASP LEU ARG LYS PHE ILE SER GLU ILE THR ILE ARG ASN ASP ASN
GACACAACAGATGTCAGATCTTAGGAAATTCATTAGTGAAATTACAATTAGAAATGATAA
CTGTGTTGTCTACAGTCTAGAATCCTTTAAGTAATCACTTTAATGTTAATCTTTACTATT
370 380 390 400 410 420

[GLN] GLU VAL [LEU] PRO GLN ARG ILE THR HIS ASP [VAL] GLY ILE LYS PRO LEU ASN PRO ASP
TCAAGAAGTGCTGCCACAAAGAATAACACATGATGTGGGTATAAAACCTTTAAATCCAGA
AGTTCTTCACGACGGTGTTTCTTATTGTGTACTACACCCATATTTTGGAAATTTAGGTCT
430 440 450 460 470 480

ASP PHE TRP ARG CYS THR SER GLY LEU PRO SER LEU MET LYS THR PRO LYS ILE ARG LEU
TGATTTTTGGAGATGCACGTCTGGTCTTCCATCTTTAATGAAAACTCCAAAAAATAAGGTT
ACTAAAAACCTCTACGTGCAGACCAGAAGGTAGAAATTACTTTTGAGGTTTTTATTCCAA
490 500 510 520 530 540

MET PRO GLY PRO GLY LEU LEU ALA MET PRO THR THR VAL ASP GLY CYS [ILE] ARG THR PRO
AATGCCAGGGCCGGGATTATTAGCTATGCCAACGACTGTTGATGGCTGTATCAGAACTCC
TTACGGTCCCGGCCCTAATAATCGATACGGTTGCTGACAACTACCGACATAGTCTTGAGG
550 560 570 580 590 600

SER LEU VAL ILE ASN ASP LEU ILE TYR ALA TYR THR SER ASN LEU ILE THR ARG GLY CYS
GTCCTTAGTTATAAATGATCTGATTTATGCTTATACCTCAAATCTAATTACTCGAGGTTG
CAGGAATCAATATTTACTAGACTAAATACGAATATGGAGTTTAGATTAATGAGCTCCAAC
610 620 630 640 650 660

GLN ASP ILE GLY LYS SER TYR GLN VAL LEU GLN ILE GLY ILE ILE THR VAL ASN SER ASP
TCAGGATATAGGAAAATCATATCAAGTCTTACAGATAGGGATAATAACTGTAAACTCAGA
AGTCCTATATCCTTTTAGTATAGTTCAGAATGTCTATCCCTATTATTGACATTTGAGTCT
670 680 690 700 710 720

LEU VAL PRO ASP LEU ASN PRO ARG ILE SER HIS THR PHE ASN ILE ASN ASP ASN ARG LYS
CTTGGTACCTGACTTAAATCCCAGGATCTCTCATACTTTTAACATAAATGACAATAGGAA
GAACCATGGACTGAATTTAGGGTCCTAGAGAGTATGAAAATTGTATTTACTGTTATCCTT
730 740 750 760 770 780

FIG.3B.

```
   SRE CYS SER LEU ALA LEU LEU ASN THR ASP VAL TYR GLN LEU CYS SER THR PRO LYS VAL
GTCATGTTCTCTAGCACTCCTAAATACAGATGTATATCAACTGTGTTCAACTCCCAAAGT
CAGTACAAGAGATCGTGAGGATTTATGTCTACATATAGTTGACACAAGTTGAGGGTTTCA
          790        800        810        820        830        840

   ASP GLU ARG SER ASP TYR ALA SER SER GLY ILE GLU ASP ILE VAL LEU ASP ILE VAL ASN
TGATGAAAGATCAGATTATGCATCATCAGGCATAGAAGATATTGTACTTGATATTGTCAA
ACTACTTTCTAGTCTAATACGTAGTAGTCCGTATCTTCTATAACATGAACTATAACAGTT
          850        860        870        880        890        900

   [TYR] ASP GLY SER ILE SER THR THR ARG PHE LYS ASN ASN ASN ILE SER PHE ASP GLN PRO
TTATGATGGCTCAATCTCAACAACAAGATTTAAGAATAATAACATAAGCTTTGATCAACC
AATACTACCGAGTTAGAGTTGTTGTTCTAAATTCTTATTATTGTATTCGAAACTAGTTGG
          910        920        930        940        950        960

   TYR ALA ALA LEU TYR PRO SER VAL GLY PRO GLY ILE TYR TYR LYS GLY LYS ILE ILE PHE
TTATGCTGCACTATACCCATCTGTTGGACCAGGGATATACTACAAAGGCAAAAATAATATT
AATACGACGTGATATGGGTAGACAACCTGGTCCCTATATGATGTTTCCGTTTTTATTATAA
          970        980        990        1000       1010       1020

   LEU GLY TYR GLY GLY LEU GLU HIS PRO ILE ASN GLU ASN [VAL] ILE CYS ASN THR THR GLY
TCTCGGGTATGGAGGTCTTGAACATCCAATAAATGAGAATGTAATCTGCAACACAACTGG
AGAGCCCATACCTCCAGAACTTGTAGGTTATTTACTCTTACATTAGACGTTGTGTTGACC
          1030       1040       1050       1060       1070       1080

   CYS PRO GLY LYS THR GLN ARG ASP CYS ASN GLN ALA SER HIS SER PRO TRP PHE SER ASP
GTGTCCCGGGAAAACACAGAGAGACTGCAATCAGGCATCTCATAGTCCATGGTTTTCAGA
CACAGGGCCCTTTTGTGTCTCTCTGACGTTAGTCCGTAGAGTATCAGGTACCAAAAGTCT
          1090       1100       1110       1120       1130       1140

   ARG ARG MET VAL ASN SER ILE ILE VAL VAL ASP LYS GLY LEU ASN SER ILE PRO LYS LEU
TAGGAGGATGGTCAACTCTATCATTGTTGTTGACAAAGGCTTAAACTCAATTCCAAAATT
ATCCTCCTACCAGTTGAGATAGTAACAACAACTGTTTCCGAATTTGAGTTAAGGTTTTAA
          1150       1160       1170       1180       1190       1200
```

FIG.3C.

LYS VAL TRP THR ILE SER MET ARG GLN ASN TYR TRP GLY SER GLU GLY ARG LEU LEU LEU
GAAGGTATGGACGATATCTATGAGACAGAATTACTGGGGGTCAGAAGGAAGGTTACTTCT
CTTCCATACCTGCTATAGATACTCTGTCTTAATGACCCCCAGTCTTCCTTCCAATGAAGA
1210 1220 1230 1240 1250 1260

LEU GLY ASN LYS ILE TYR ILE TYR THR ARG SER THR SER TRP HIS SER LYS LEU GLN LEU
ACTAGGTAACAAGATCTATATATATACAAGATCCACAAGTTGGCATAGCAAGTTACAATT
TGATCCATTGTTCTAGATATATATATGTTCTAGGTGTTCAACCGTATCGTTCAATGTTAA
1270 1280 1290 1300 1310 1320

GLY ILE ILE ASP ILE THR ASP TYR SER ASP ILE ARG ILE LYS TRP THR TRP HIS ASN VAL
AGGAATAATTGATATTACTGATTACAGTGATATAAGGATAAAATGGACATGGCATAATGT
TCCTTATTAACTATAATGACTAATGTCACTATATTCCTATTTTACCTGTACCGTATTACA
1330 1340 1350 1360 1370 1380

LEU SER ARG PRO GLY ASN ASN GLU CYS PRO TRP GLY HIS SER CYS PRO ASP GLY CYS ILE
GCTATCAAGACCAGGAAACAATGAATGTCCATGGGGACATTCATGTCCAGATGGATGTAT
CGATAGTTCTGGTCCTTTGTTACTTACAGGTACCCCTGTAAGTACAGGTCTACCTACATA
1390 1400 1410 1420 1430 1440

THR GLY VAL TYR THR ASP ALA TYR PRO LEU ASN PRO THR GLY SER ILE VAL SER SER VAL
AACAGGAGTATATACTGATGCATATCCACTCAATCCCACAGGGAGCATTGTGTCATCTGT
TTGTCCTCATATATGACTACGTATAGGTGAGTTAGGGTGTCCCTCGTAACACAGTAGACA
1450 1460 1470 1480 1490 1500

ILE LEU ASP SER GLN LYS SER ARG VAL ASN PRO VAL ILE THR TYR SER THR ALA THR GLU
CATATTAGATTCACAAAAATCGAGAGTGAACCCAGTCATAACTTACTCAACAGCAACCGA
GTATAATCTAAGTGTTTTTAGCTCTCACTTGGGTCAGTATTGAATGAGTTGTCGTTGGCT
1510 1520 1530 1540 1550 1560

ARG VAL ASN GLU LEU ALA ILE ARG ASN ARG THR LEU SER ALA GLY TYR THR THR THR SER
AAGAGTAAACGAGCTGGCCATCCGAAACAGAACACTCTCAGCTGGATATACAACAACAAG
TTCTCATTTGCTCGACCGGTAGGCTTTGTCTTGTGAGAGTCGACCTATATGTTGTTGTTC
1570 1580 1590 1600 1610 1620

FIG.3D.

```
  CYS  ILE  THR  HIS  TYR  ASN  LYS  GLY  TYR  CYS  PHE  HIS  ILE  VAL  GLU  ILE  ASN [GLN] LYS  SER
CTGCATCACACACTATAACAAAGGATATTGTTTTCATATAGTAGAAATAAATCAGAAAAG
GACGTAGTGTGTGATATTGTTTCCTATAACAAAAGTATATCATCTTTATTTAGTCTTTTC
          1630          1640          1650          1660          1670          1680

  LEU [ASN] THR [LEU] GLN  PRO  MET  LEU  PHE  LYS  THR  GLU [VAL] PRO  LYS  SER  CYS  SER  ***
CTTAAACACACTTCAACCCATGTTGTTCAAGACAGAGGTTCCAAAAAGCTGCAGTTAATC
GAATTTGTGTGAAGTTGGGTACAACAAGTTCTGTCTCCAAGGTTTTTCGACGTCAATTAG
          1690          1700          1710          1720          1730          1740

ATAATTAACCGCAATATGCATTAACCTATCTATAATACAAGTATATGATAAGTAATCAGC
TATTAATTGGCGTTATACGTAATTGGATAGATATTATGTTCATATACTATTCATTAGTCG
          1750          1760          1770          1780          1790          1800

AATCAGACAATAGACAAAAGGGAAATATAAAAA
TTAGTCTGTTATCTGTTTTCCCTTTATATTTTT
          1810          1820          1830
```

NUCLEOTIDE SEQUENCE OF THE PIV-3 HN GENE. THE cDNA SEQUENCE IS SHOWN IN THE PLUS (mRNA) STRAND SENSE IN THE 5' TO 3' DIRECTION. THE TRANSMEMBRANE (TM) ANCHOR DOMAIN IS UNDERLINED. AMINO ACIDS DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY THE PIV-3 HN GENE ARE BOXED.

FIG.3E.

RESTRICTION MAP OF THE PIV-3 HN GENE

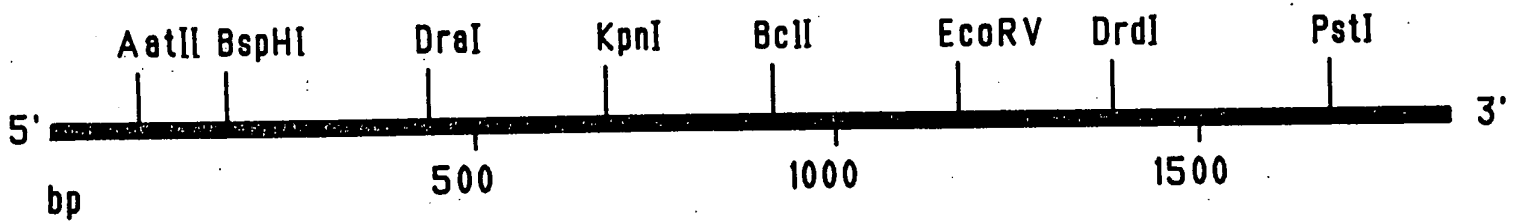
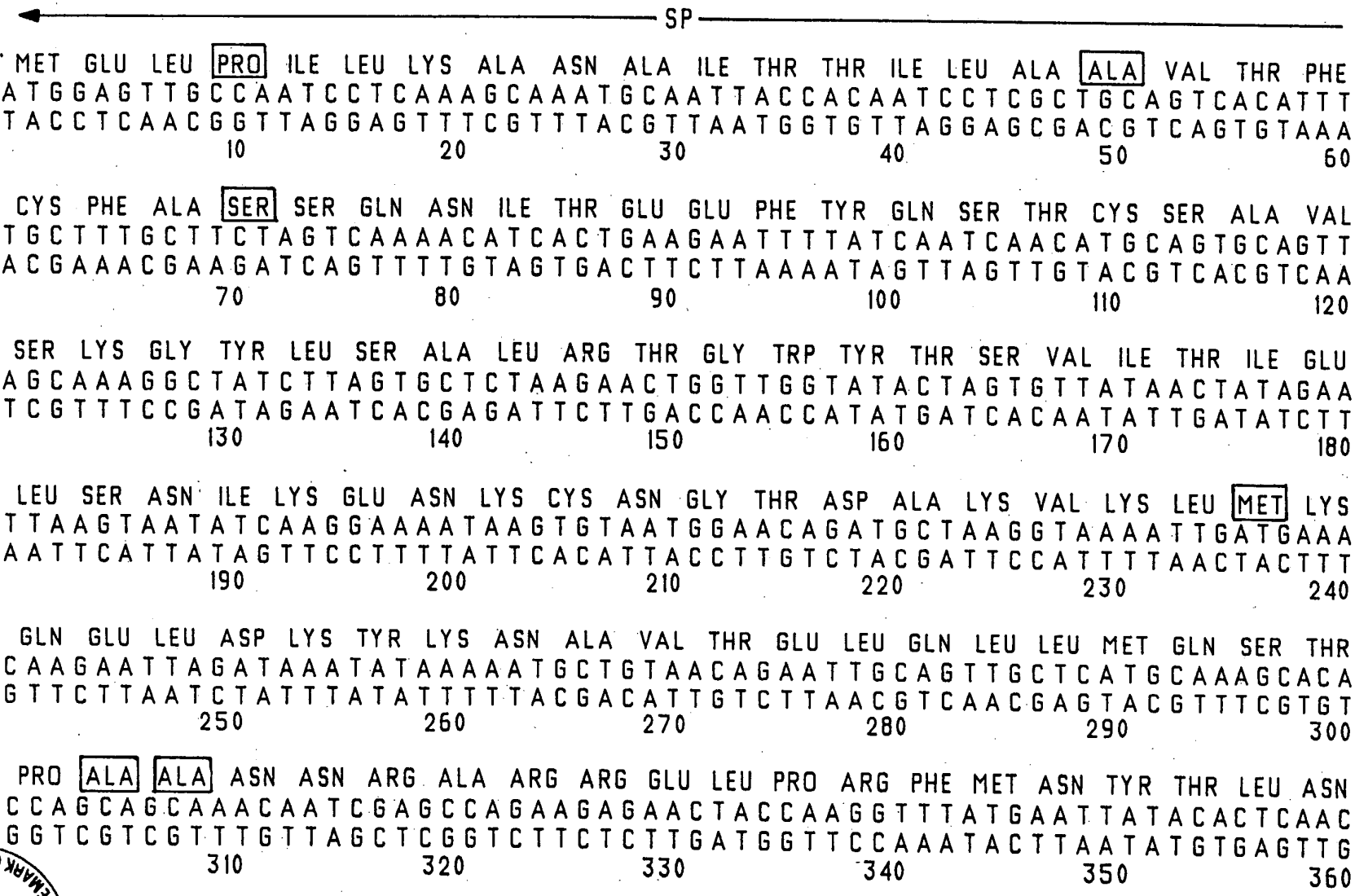


FIG.4.

FIG.5A. NUCLEOTIDE SEQUENCE OF THE RSV F GENE.



F2-F1 CLEAVAGE SITE

```
ASN [THR] LYS LYS THR ASN VAL THR LEU SER LYS LYS ARG LYS ARG ARG↓PHE LEU GLY PHE
AATACCAAAAAAACCAATGTAACATTAAGCAAGAAAAGGAAAAGAAGATTTCTTGGTTTT
TTATGGTTTTTTTGGTTACATTGTAATTCGTTCTTTTCCTTTTCTTCTAAAGAACCAAAA
          370        380        390        400        410        420

LEU LEU GLY VAL GLY SER ALA ILE ALA SER GLY [ILE] ALA VAL SER LYS VAL LEU HIS LEU
TTGTTAGGTGTTGGATCTGCAATCGCCAGTGGCATTGCTGTATCTAAGGTCCTGCACTTA
AACAATCCACAACCTAGACGTTAGCGGTCACCGTAACGACATAGATTCCAGGACGTGAAT
          430        440        450        460        470        480

GLU GLY GLU VAL ASN LYS ILE LYS SER ALA LEU LEU SER THR ASN LYS ALA VAL VAL SER
GAAGGAGAAGTGAACAAGATCAAAAGTGCTCTACTATCCACAAACAAGGCCGTAGTCAGT
CTTCCTCTTCACTTGTTCTAGTTTTCACGAGATGATAGGTGTTTGTTCCGGCATCAGTCA
          490        500        510        520        530        540

LEU SER ASN GLY VAL SER VAL LEU THR SER LYS VAL LEU ASP LEU LYS ASN TYR ILE ASP
TTATCAAATGGAGTTAGTGTCTTAACCAGCAAAGTGTTAGACCTCAAAAACTATATAGAT
AATAGTTTACCTCAATCACAGAATTGGTCGTTTCACAATCTGGAGTTTTTGATATATCTA
          550        560        570        580        590        600

LYS GLN LEU LEU PRO ILE VAL ASN LYS ARG SER CYS [ARG] ILE SER ASN ILE GLU THR VAL
AAACAATTGTTACCTATTGTGAATAAGCGAAGCTGCAGAATATCAAATATAGAAACTGTG
TTTGTTAACAATGGATAACACTTATTCGCTTCGACGTCTTATAGTTTATATCTTTGACAC
          610        620        630        640        650        660

ILE GLU PHE GLN HIS LYS ASN ASN ARG LEU LEU GLU ILE THR ARG GLU PHE SER VAL ASN
ATAGAGTTCCAACACAAGAACAACAGACTACTAGAGATTACCAGGGAATTTAGTGTTAAT
TATCTCAAGGTTGTGTTCTTGTTGTCTGATGATCTCTAATGGTCCCTTAAATCACAATTA
          670        680        690        700        710        720

ALA GLY VAL THR THR PRO VAL SER THR TYR MET LEU THR ASN SER GLU LEU LEU SER LEU
GCAGGTGTAACTACACCTGTAAGCACTTACATGTTAACTAATAGTGAATTATTGTCATTA
CGTCCACATTGATGTGGACATTCGTGAATGTACAATTGATTATCACTTAATAACAGTAAT
          730        740        750        760        770        780
```

FIG.5B.

ILE ASN ASP MET PRO ILE THR ASN ASP GLN LYS LYS LEU MET SER ASN ASN VAL GLN ILE
ATCAATGATATGCCTATAACAAATGATCAGAAAAAGTTAATGTCCAACAATGTTCAAATA
TAGTTACTATACGGATATTGTTTACTAGTCTTTTTCAATTACAGGTTGTTACAAGTTTAT
790 800 810 820 830 840

VAL ARG GLN GLN SER TYR SER ILE MET SER ILE ILE LYS GLU GLU VAL LEU ALA TYR VAL
GTTAGACAGCAAAGTTACTCTATCATGTCCATAATAAAAGAGGAAGTCTTAGCATATGTA
CAATCTGTCGTTTCAATGAGATAGTACAGGTATTATTTTCTCCTTCAGAATCGTATACAT
850 860 870 880 890 900

VAL GLN LEU PRO LEU TYR GLY VAL ILE ASP THR PRO CYS TRP LYS LEU HIS THR SER PRO
GTACAATTACCACTATATGGTGTGATAGATACACCTTGTTGGAAATTACACACATCCCCT
CATGTTAATGGTGATATACCACACTATCTATGTGGAACAACCTTTAATGTGTGTAGGGGA
910 920 930 940 950 960

LEU CYS THR THR ASN THR LYS GLU GLY SER ASN ILE CYS LEU THR ARG THR ASP ARG GLY
CTATGTACAACCAACACAAAAGAAGGGTCAAACATCTGTTTAACAAGAACTGACAGAGGA
GATACATGTTGGTTGTGTTTTCTTCCCAGTTTGTAGACAAATTGTTCTTGACTGTCTCCT
970 980 990 1000 1010 1020

TRP TYR CYS ASP ASN ALA GLY SER VAL SER PHE PHE PRO GLN ALA GLU THR CYS LYS VAL
TGGTACTGTGACAATGCAGGATCAGTATCTTTCTTCCCACAAGCTGAAACATGTAAAGTT
ACCATGACACTGTTACGTCCTAGTCATAGAAAGAAGGGTGTTCGACTTTGTACATTTCAA
1030 1040 1050 1060 1070 1080

GLN SER ASN ARG VAL PHE CYS ASP THR MET ASN SER LEU THR LEU PRO SER GLU VAL ASN
CAATCGAATCGAGTATTTTGTGACACAATGAACAGTTTAACATTACCAAGTGAAGTAAAT
GTTAGCTTAGCTCATAAAACACTGTGTTACTTGTCAAATTGTAATGGTTCACTTCATTTA
1090 1100 1110 1120 1130 1140

LEU CYS ASN VAL ASP ILE PHE ASN PRO LYS TYR ASP CYS LYS ILE MET THR SER LYS THR
CTCTGCAATGTTGACATATTCAATCCCAAATATGATTGTAAAATTATGACTTCAAAAACA
GAGACGTTACAACTGTATAAGTTAGGGTTTATACTAACATTTTAATACTGAAGTTTTTGT
1150 1160 1170 1180 1190 1200

FIG.5C.

ASP VAL SER SER SER VAL ILE THR SER LEU GLY ALA ILE VAL SER CYS TYR GLY LYS THR
GATGTAAGCAGCTCCGTTATCACATCTCTAGGAGCCATTGTGTCATGCTATGGCAAAACT
CTACATTCGTCGAGGCAATAGTGTAGAGATCCTCGGTAACACAGTACGATACCGTTTTGA
1210 1220 1230 1240 1250 1260

LYS CYS THR ALA SER ASN LYS ASN ARG GLY ILE ILE LYS THR PHE SER ASN GLY CYS ASP
AAATGTACAGCATCCAATAAAAATCGTGGAATCATAAAGACATTTTCTAACGGGTGTGAT
TTTACATGTCGTAGGTTATTTTTAGCACCTTAGTATTTCTGTAAAAGATTGCCCACACTA
1270 1280 1290 1300 1310 1320

TYR VAL SER ASN LYS GLY VAL ASP THR VAL SER VAL GLY ASN THR LEU TYR TYR VAL ASN
TATGTATCAAATAAAGGGGTGGACACTGTGTCTGTAGGTAACACATTATATTATGTAAAT
ATACATAGTTTATTTCCCCACCTGTGACACAGACATCCATTGTGTAATATAATACATTTA
1330 1340 1350 1360 1370 1380

LYS GLN GLU GLY LYS SER LEU TYR VAL LYS GLY GLU PRO ILE ILE ASN PHE TYR ASP PRO
AAGCAAGAAGGCAAAAAGTCTCTATGTAAAAGGTGAACCAATAATAAATTTCTATGACCCA
TTCGTTCTTCCGTTTTCAGAGATACATTTTCCACTTGGTTATTATTTAAAGATACTGGGT
1390 1400 1410 1420 1430 1440

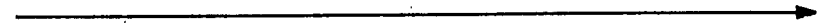
LEU VAL PHE PRO SER ASP GLU PHE ASP ALA SER ILE SER GLN VAL ASN GLU LYS ILE ASN
TTAGTATTCCCCTCTGATGAATTTGATGCATCAATATCTCAAGTCAACGAGAAGATTAAC
AATCATAAGGGGAGACTACTTAAACTACGTAGTTATAGAGTTCAGTTGCTCTTCTAATTG
1450 1460 1470 1480 1490 1500

GLN SER LEU ALA PHE ILE ARG LYS SER ASP GLU LEU LEU HIS ASN VAL ASN ALA GLY LYS
CAGAGTTTAGCATTTATTCGTAAATCCGATGAATTATTACATAATGTAAATGCTGGTAAA
GTCTCAAATCGTAAATAAGCATTTAGGCTACTTAATAATGTATTACATTTACGACCATTT
1510 1520 1530 1540 1550 1560

TM

SER THR THR ASN ILE MET ILE THR THR ILE ILE ILE GLU ILE ILE VAL ILE LEU LEU SER
TCAACCACAAATATCATGATAACTACTATAATTATAGAGATTATAGTAATATTGTTATCA
AGTTGGTGTTTATAGTACTATTGATGATATTAATATCTCTAATATCATTATAACAATAGT
1570 1580 1590 1600 1610 1620

FIG.5D.



LEU ILE ALA VAL GLY LEU LEU LEU TYR CYS LYS ALA ARG SER THR PRO VAL THR LEU SER

```
TTAATTGCTGTTGGACTGCTCCTATACTGTAAGGCCAGAAGCACACCAGTCACACTAAGC
AATTAACGACAACCTGACGAGGATATGACATTCCGGTCTTCGTGTGGTCAGTGTGATTCG
          1630        1640        1650        1660        1670        1680
```

LYS, ASP GLN LEU SER GLY ILE ASN ASN ILE ALA PHE SER ASN

```
AAGGATCAACTGAGTGGTATAAATAATATTGCATTTAGTAACTGAATAAAAATAGCACCT
TTCCTAGTTGACTCACCATATTTATTATAACGTAAATCATTGACTTATTTTTATCGTGGA
          1690        1700        1710        1720        1730        1740

AATCATGTTCTTACAATGGTTTACTATCTGCTCATAGACAACCCATCTATCATTGGATTT
TTAGTACAAGAATGTTACCAAATGATAGACGAGTATCTGTTGGGTAGATAGTAACCTAAA
          1750        1760        1770        1780        1790        1800

TCTTAAAATCTGAACTTCATCGAAACTCTTATCTATAAACCATCTCACTTACACTATTTA
AGAATTTTAGACTTGAAGTAGCTTTGAGAATAGATATTTGGTAGAGTGAATGTGATAAAT
          1810        1820        1830        1840        1850        1860

AGTAGATTCCTAGTTTATAGTTATAT  3'
TCATCTAAGGATCAAATATCAATATA
          1870        1880
```

NUCLEOTIDE SEQUENCE OF THE RSV F GENE.THE cDNA SEQUENCE IS SHOWN IN THE PLUS (mRNA) STRAND SENSE IN THE 5' TO 3' DIRECTION.THE SIGNAL PEPTIDE (SP) AND THE TRANSMEMBRANE (TM) ANCHOR DOMAIN ARE UNDERLINED.THE PREDICTED F2-F1 CLEAVAGE SITE IS INDICATED BY THE ARROW (↓).AMINO ACIDS DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY THE RSV F GENE ARE BOXED.

FIG.5E.

RESTRICTION MAP OF THE RSV F GENE

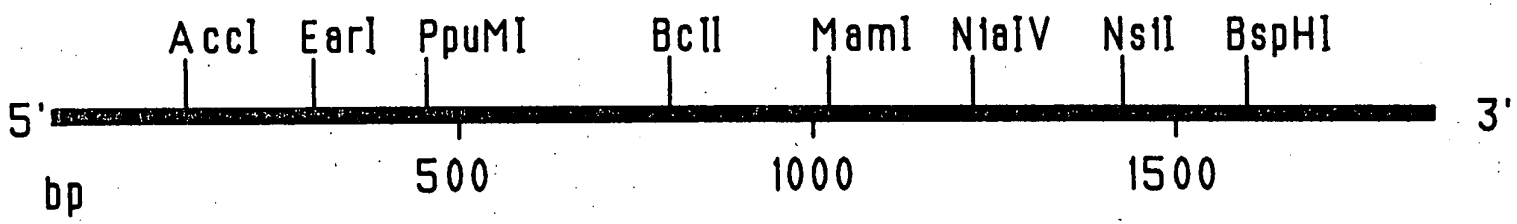


FIG.6.

FIG.7A. NUCLEOTIDE SEQUENCE OF THE RSV G GENE

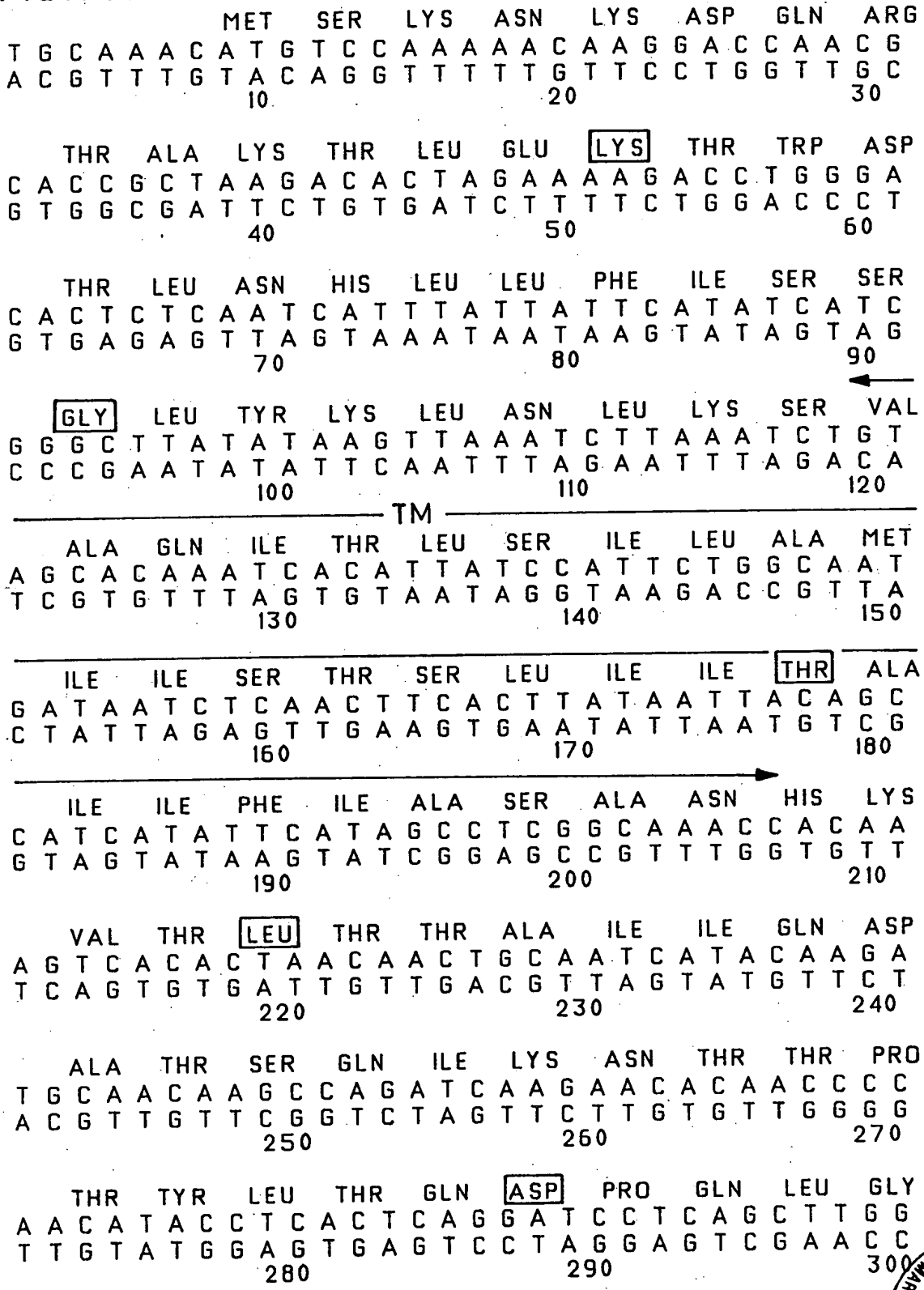


FIG.7B.

```
   ILE  SER [PHE] SER  ASN [LEU] SER  GLU  ILE  THR
A A T C A G C T T C T C C A A T C T G T C T G A A A T T A C
T T A G T C G A A G A G G T T A G A C A G A C T T T A A T G
                  310                 320                 330

   SER  GLN [THR] THR  THR  ILE  LEU  ALA  SER  THR
A T C A C A A A C C A C C A C C A T A C T A G C T T C A A C
T A G T G T T T G G T G G T G G T A T G A T C G A A G T T G
                  340                 350                 360

   THR  PRO  GLY  VAL  LYS  SER [ASN] LEU  GLN [PRO]
A A C A C C A G G A G T C A A G T C A A A C C T G C A A C C
T T G T G G T C C T C A G T T C A G T T T G G A C G T T G G
                  370                 380                 390

   THR  THR  VAL  LYS  THR  LYS  ASN  THR  THR  THR
C A C A A C A G T C A A G A C T A A A A A A C A C A A C A A C
G T G T T G T C A G T T C T G A T T T T T G T G T T G T T G
                  400                 410                 420

   THR  GLN  THR  GLN  PRO  SER  LYS  PRO  THR  THR
A A C C C A A A C A C A A C C C A G C A A G C C C A C T A C
T T G G G T T T G T G T T G G G T C G T T C G G G T G A T G
                  430                 440                 450

   LYS  GLN  ARG  GLN  ASN  LYS  PRO  PRO [ASN] LYS
A A A A C A A C G C C A A A A C A A A C C A C C A A A C A A
T T T T G T T G C G G T T T T G T T T G G T G G T T T G T T
                  460                 470                 480

   PRO  ASN  ASN  ASP  PHE  HIS  PHE  GLU  VAL  PHE
A C C C A A T A A T G A T T T T C A C T T C G A A G T G T T
T G G G T T A T T A C T A A A A G T G A A G C T T C A C A A
                  490                 500                 510

   ASN  PHE  VAL  PRO  CYS  SER  ILE  CYS  SER  ASN
T A A C T T T G T A C C C T G C A G C A T A T G C A G C A A
A T T G A A A C A T G G G A C G T C G T A T A C G T C G T T
                  520                 530                 540

   ASN  PRO  THR  CYS  TRP  ALA  ILE  CYS  LYS  ARG
C A A T C C A A C C T G C T G G G C T A T C T G C A A A A G
G T T A G G T T G G A C G A C C C G A T A G A C G T T T T C
                  550                 560                 570

   ILE  PRO  ASN  LYS  LYS  PRO  GLY  LYS  LYS  THR
A A T A C C A A A C A A A A A A C C A G G A A A G A A A A C
T T A T G G T T T G T T T T T T G G T C C T T T C T T T T G
                  580                 590                 600
```

FIG.7C.

```
    THR  THR  LYS  PRO  THR  LYS  LYS  PRO  THR  PHE
C A C C A C C A A G C C T A C A A A A A A A C C A A C C T T
G T G G T G G T T C G G A T G T T T T T T T G G T T G G A A
                610                  620                  630

    LYS  THR  THR  LYS  LYS  ASP  LEU  LYS  PRO  GLN
C A A G A C A A C C A A A A A A G A T C T C A A A C C T C A
G T T C T G T T G G T T T T T T C T A G A G T T T G G A G T
                640                  650                  660

    THR  THR  LYS  PRO  LYS  GLU  VAL  PRO  THR  THR
A A C C A C T A A A C C A A A G G A A G T A C C C A C C A C
T T G G T G A T T T G G T T T C C T T C A T G G G T G G T G
                670                  680                  690

    LYS  PRO  THR  GLU  GLU  PRO  THR  ILE  ASN  THR
C A A G C C C A C A G A A G A G C C A A C C A T C A A C A C
G T T C G G G T G T C T T C T C G G T T G G T A G T T G T G
                700                  710                  720

    THR  LYS  THR  ASN  ILE  THR  THR  THR  LEU  LEU
C A C C A A A A C A A A C A T C A C A A C T A C A C T G C T
G T G G T T T T G T T T G T A G T G T T G A T G T G A C G A
                730                  740                  750

    THR  ASN  ASN  THR  THR  GLY  ASN  PRO  LYS  LEU
C A C C A A C A A C A C C A C A G G A A A T C C A A A A C T
G T G G T T G T T G T G G T G T C C T T T A G G T T T T G A
                760                  770                  780

    THR  SER  GLN  MET  GLU  THR  PHE  HIS  SER  THR
C A C A A G T C A A A T G G A A A C C T T C C A C T C A A C
G T G T T C A G T T T A C C T T T G G A A G G T G A G T T G
                790                  800                  810

    SER  SER  GLU  GLY  ASN  LEU  SER  PRO  SER  GLN
C T C C T C C G A A G G C A A T C T A A G C C C T T C T C A
G A G G A G G C T T C C G T T A G A T T C G G G A A G A G T
                820                  830                  840

    VAL  SER  THR  THR  SER  GLU  HIS  PRO  SER  GLN
A G T C T C C A C A A C A T C C G A G C A C C C A T C A C A
T C A G A G G T G T T G T A G G C T C G T G G G T A G T G T
                850                  860                  870

    PRO  SER  SER  PRO  PRO  ASN  THR  THR  ARG  GLN
A C C C T C A T C T C C A C C C A A C A C A A C A C G C C A
T G G G A G T A G A G G T G G G T T G T G T T G T G C G G T
                880                  890                  900
```

NUCLEOTIDE SEQUENCE OF THE RSV G GENE. THE cDNA SEQUENCE IS SHOWN IN THE PLUS (mRNA) STRAND SENSE IN THE 5' TO 3' DIRECTION. THE TRANSMEMBRANE (TM) ANCHOR DOMAIN IS UNDERLINED. AMINO ACIDS DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY THE RSV G GENE ARE BOXED.

FIG.7D.

RESTRICTION MAP OF RSV G GENE

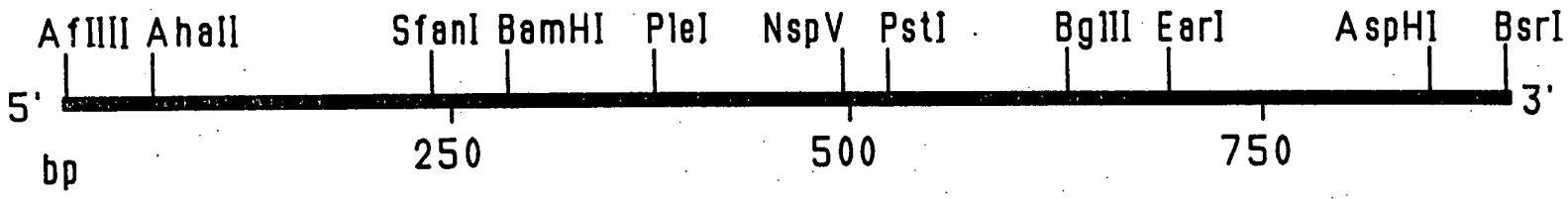


FIG.8.

Construction of a Bluescript-based expression vector containing the chimeric F_{PIV-3} -F_{RSV} gene with the 5' untranslated region of the PIV-3 F gene intact but lacking the nucleotide sequences coding for the hydrophobic anchor domains and cytoplasmic tails of both the PIV-3 and RSV F genes.

Step 1: Preparation of the plasmid containing the modified PIV-3 F gene

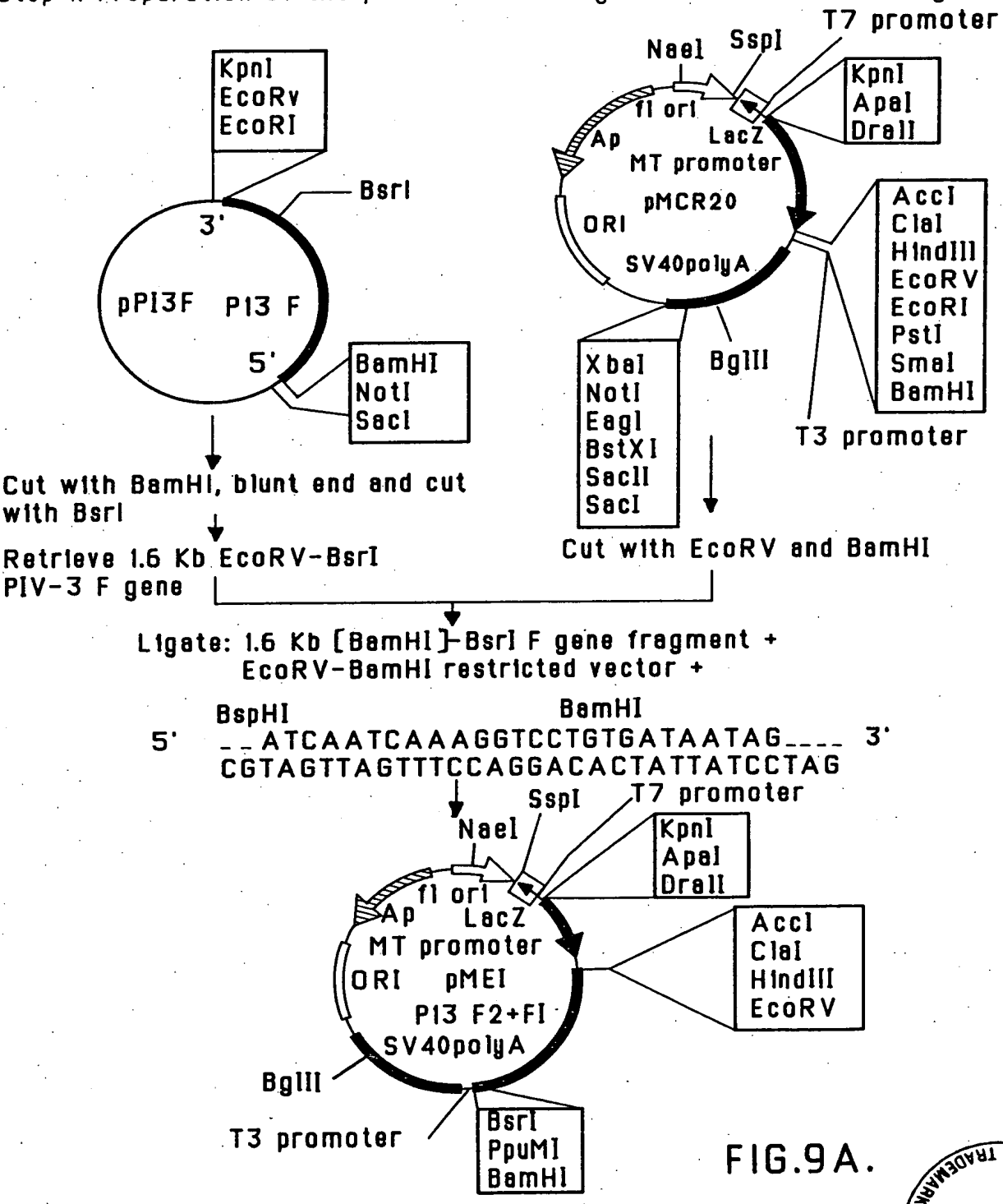
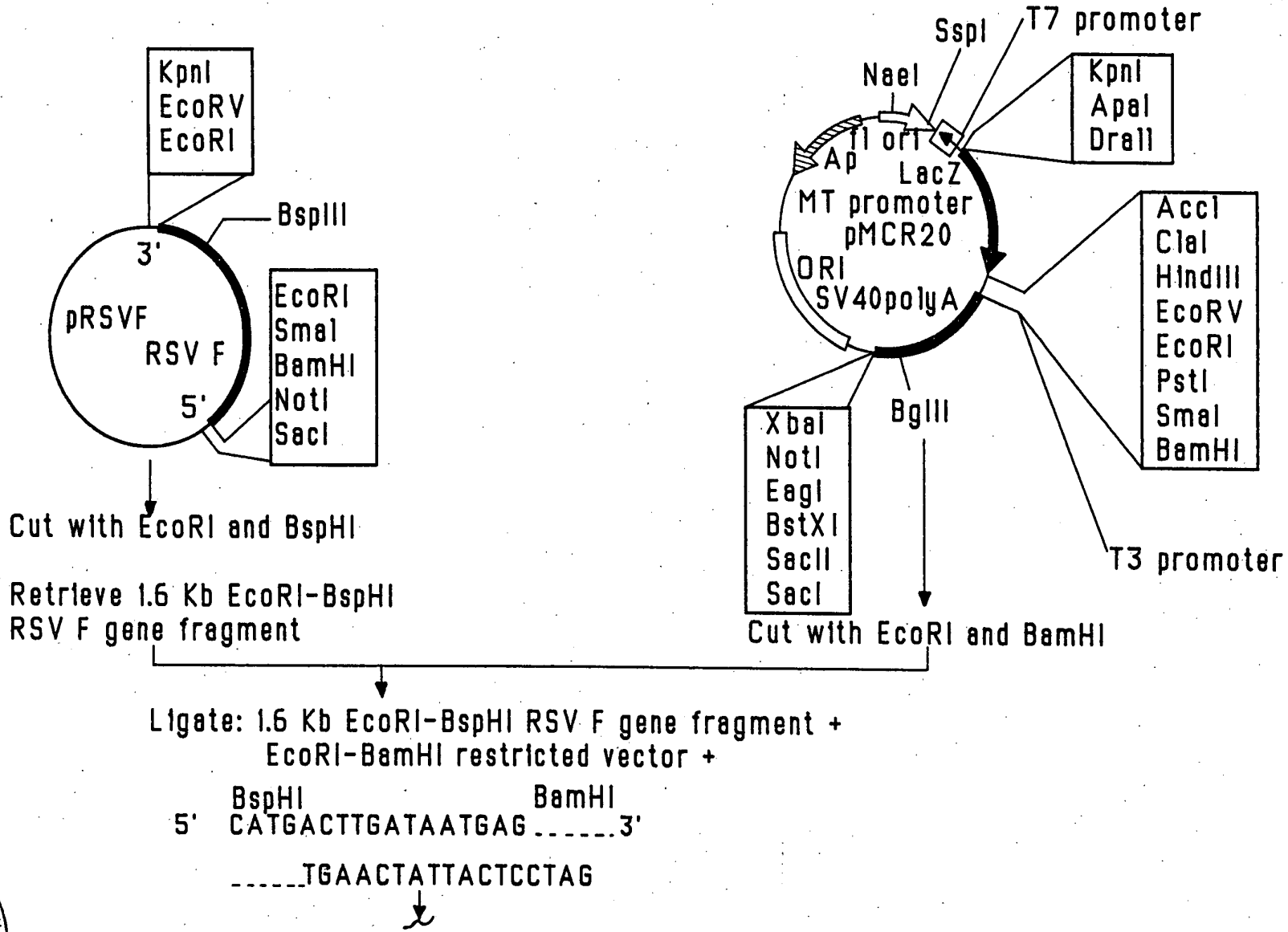


FIG.9A.

FIG.9B. Step 2: Preparation of the plasmid containing the modified RSV F gene



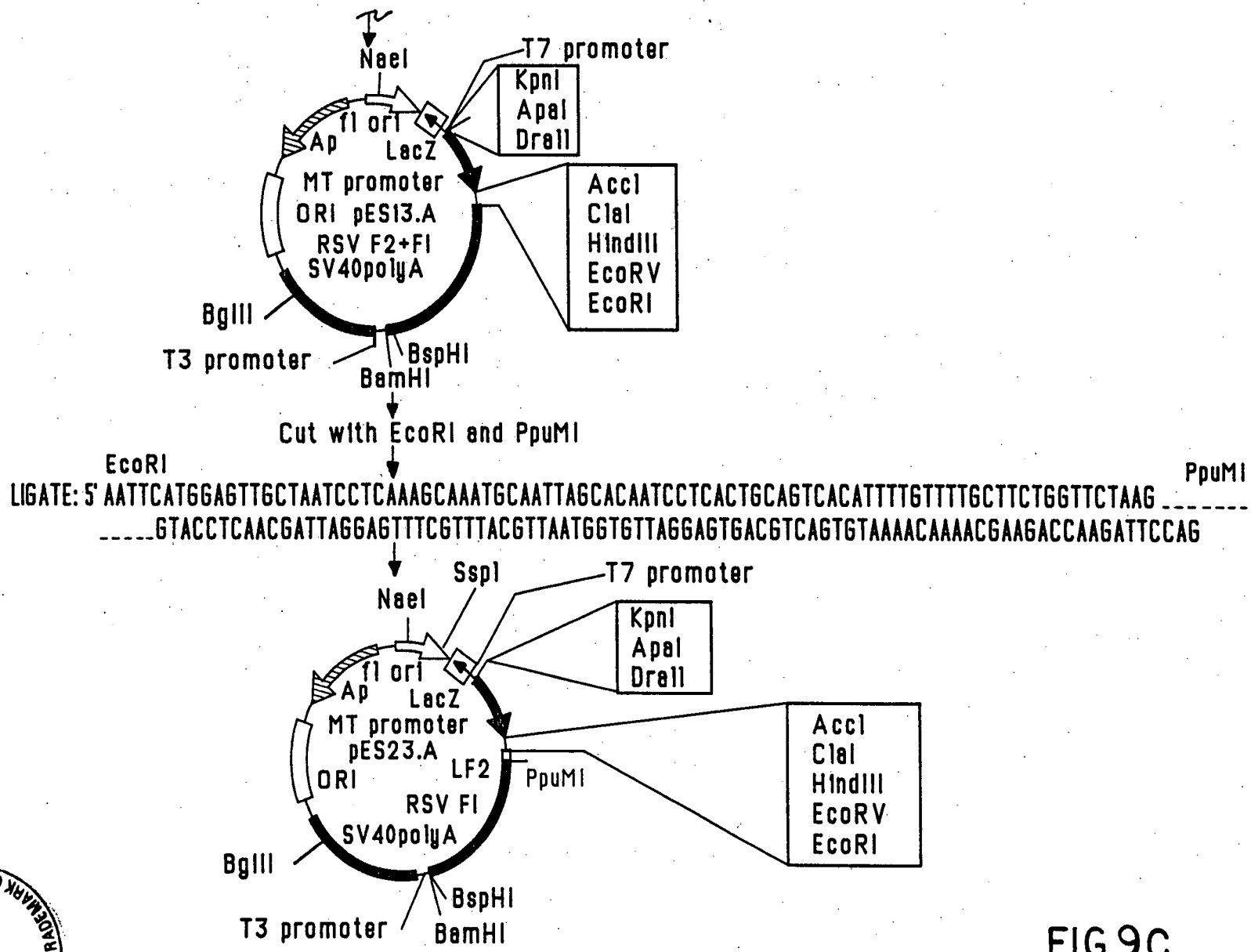
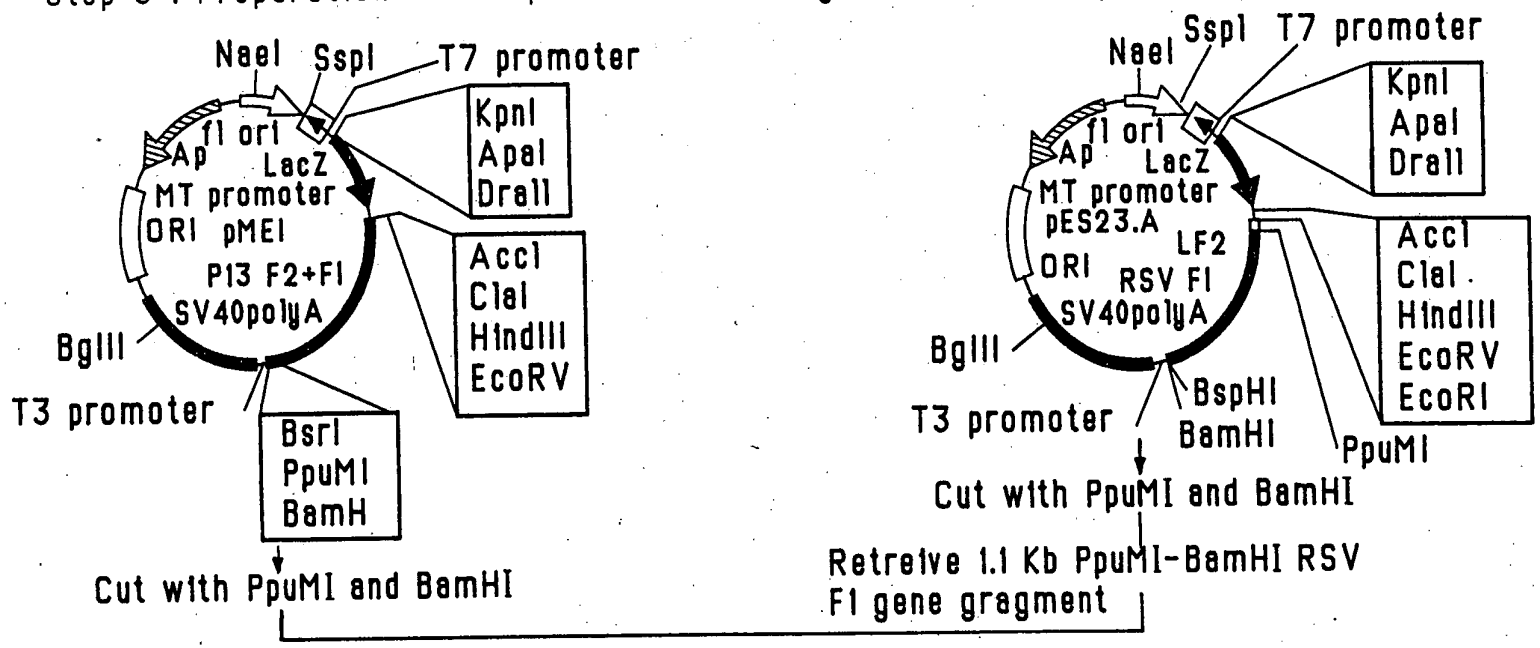


FIG.9C.

Step 3 : Preparation of the plasmid containing the chimeric F_{PIV-3} -F_{RSV} gene



Ligate: PpuMI-BamHI restricted vector +
1.1 Kd PpuMI-BamHI RSV F1 fragment

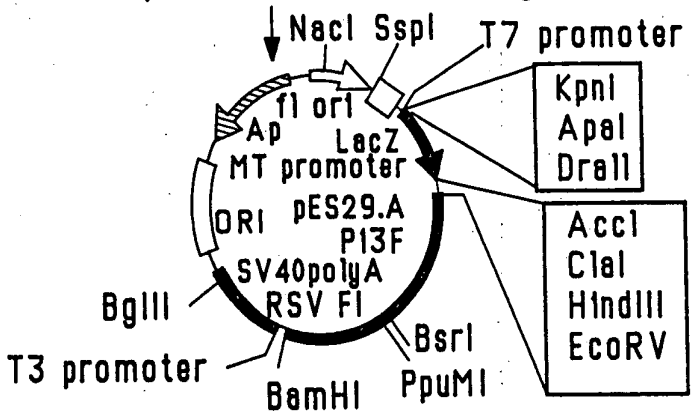


FIG.9D.

Construction of a Bluescript-based expression vector containing the PIV-3 F gene lacking the 5' untranslated sequence and transmembrane anchor and cytoplasmic tail coding regions.

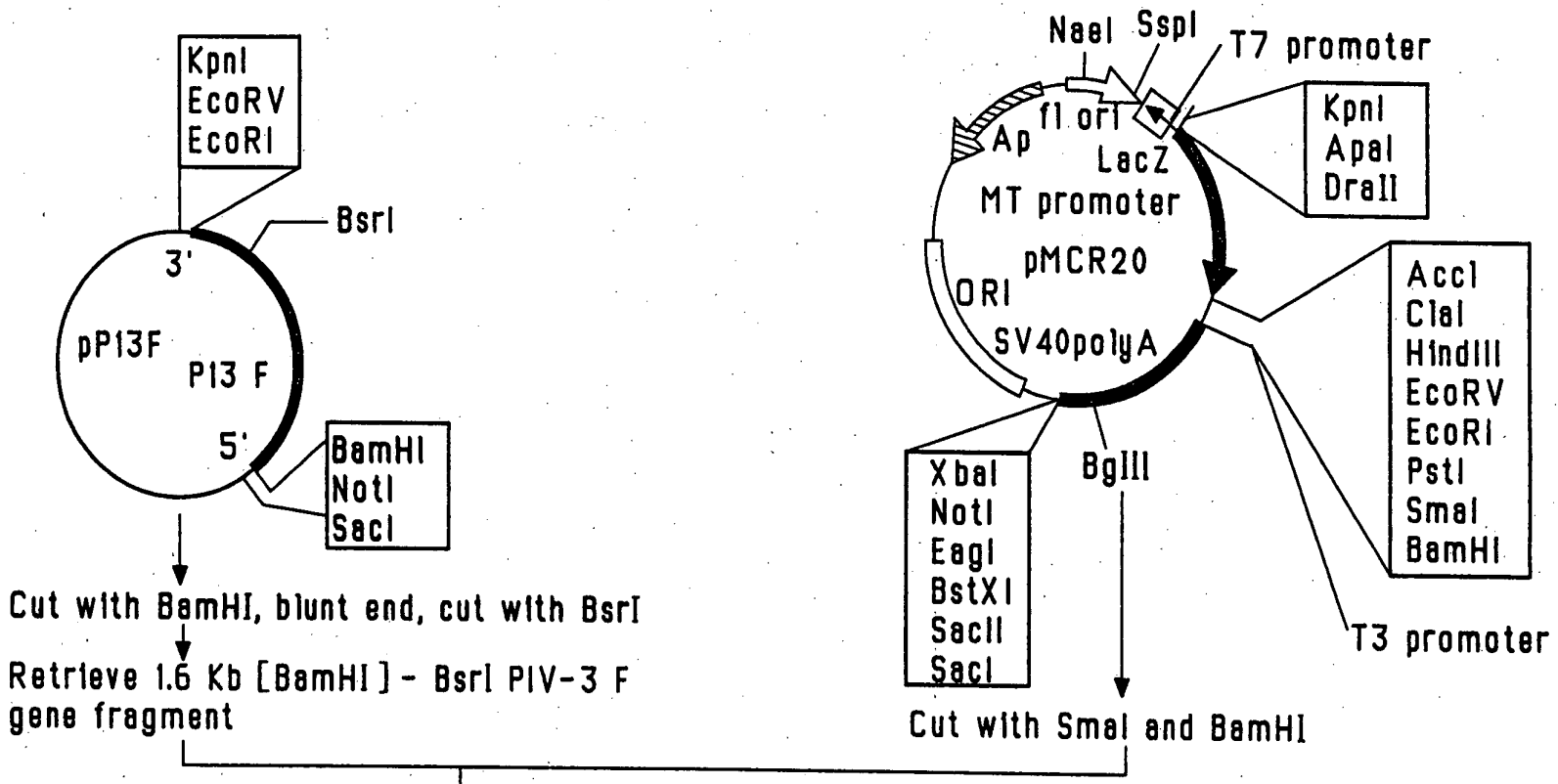
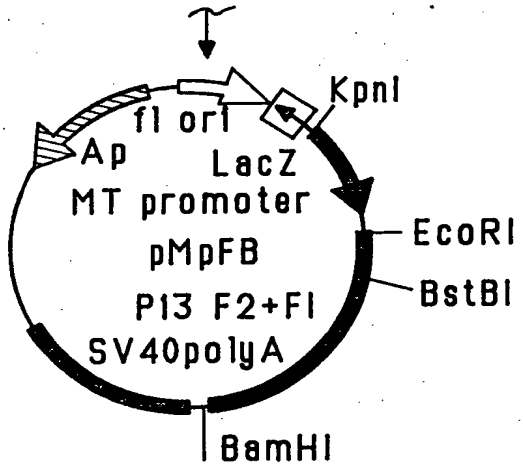


FIG.10A.

FIG.10B.



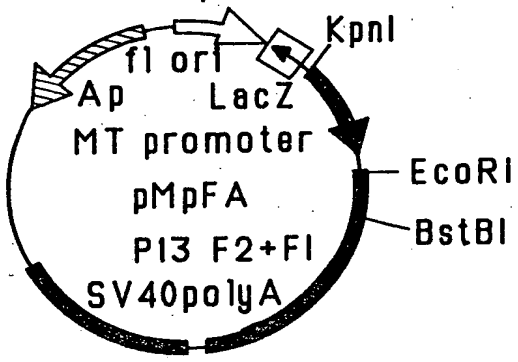
Cut with EcoRI and BstBI

Retreive: EcoRI-BstBI restricted vector

Ligate: EcoRI-BstBI restricted vector +

EcoRI PpuMI

AATTCATGCCAACTTTAATACTGCTAATTATTACAACAATGATTATGG
CATCTTCCTGCCAAATAGATATCACAAAACTACAGCAATGTAGGTGTA
TTGGTCAACAGTCCCAAAGGGATGAAGATATCACAAAACTT____ 3'
____GTACGGTTGAAATTATGACGATTAATAATGTTGTTACTAATACC
GTAGAAGGACGGTTTATCTATAGTGTTTTGATGTCGTACATCCACATA
ACCAGTTGTCAGGGTTTCCCTACTTCTATAGTGTTTTGAAGCTT



Construction of the chimeric F_{PIV-3}-F_{RSV} gene consisting of the truncated PIV-3 F gene devoid of the 5' untranslated region linked to the truncated RSV F1 gene.

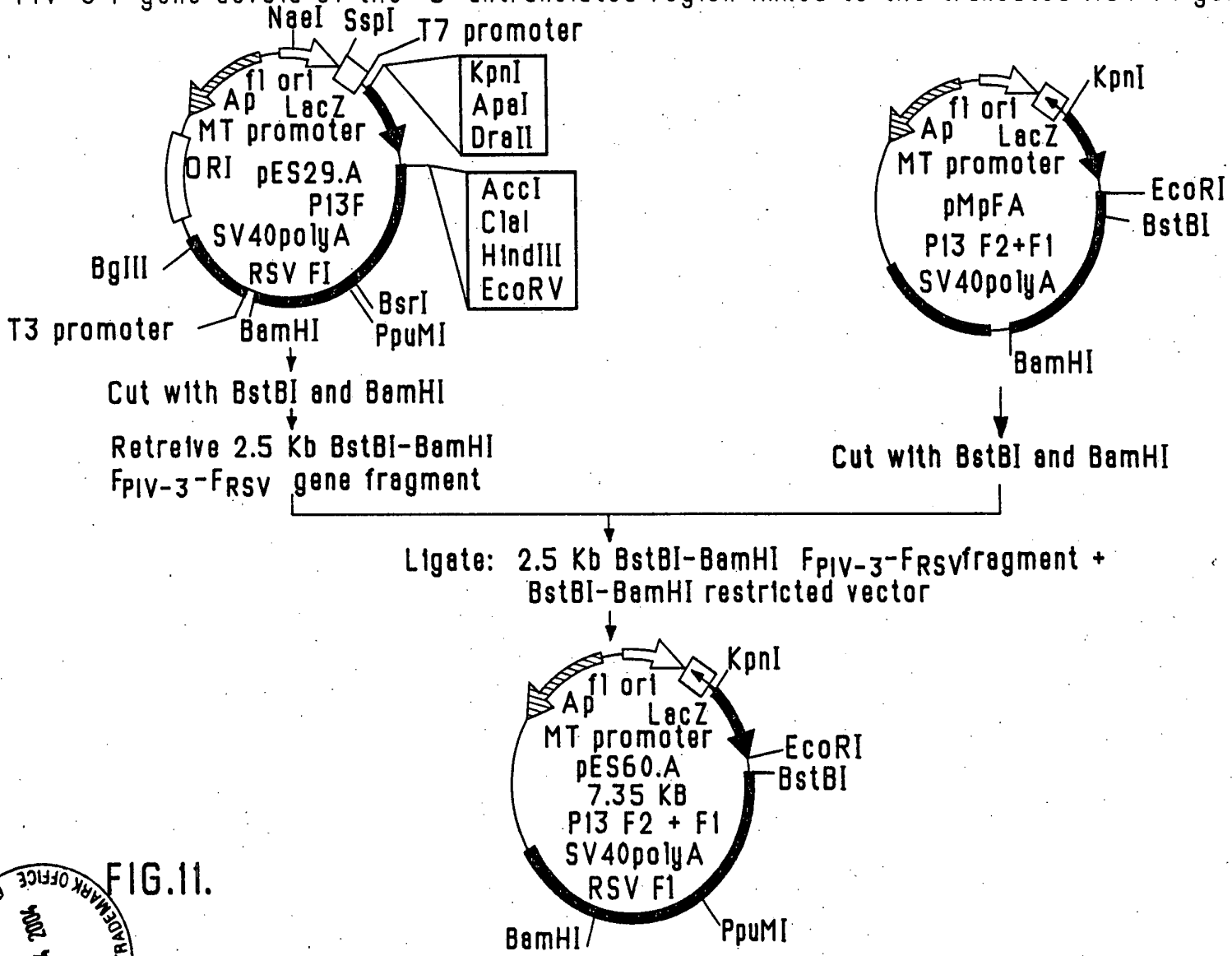
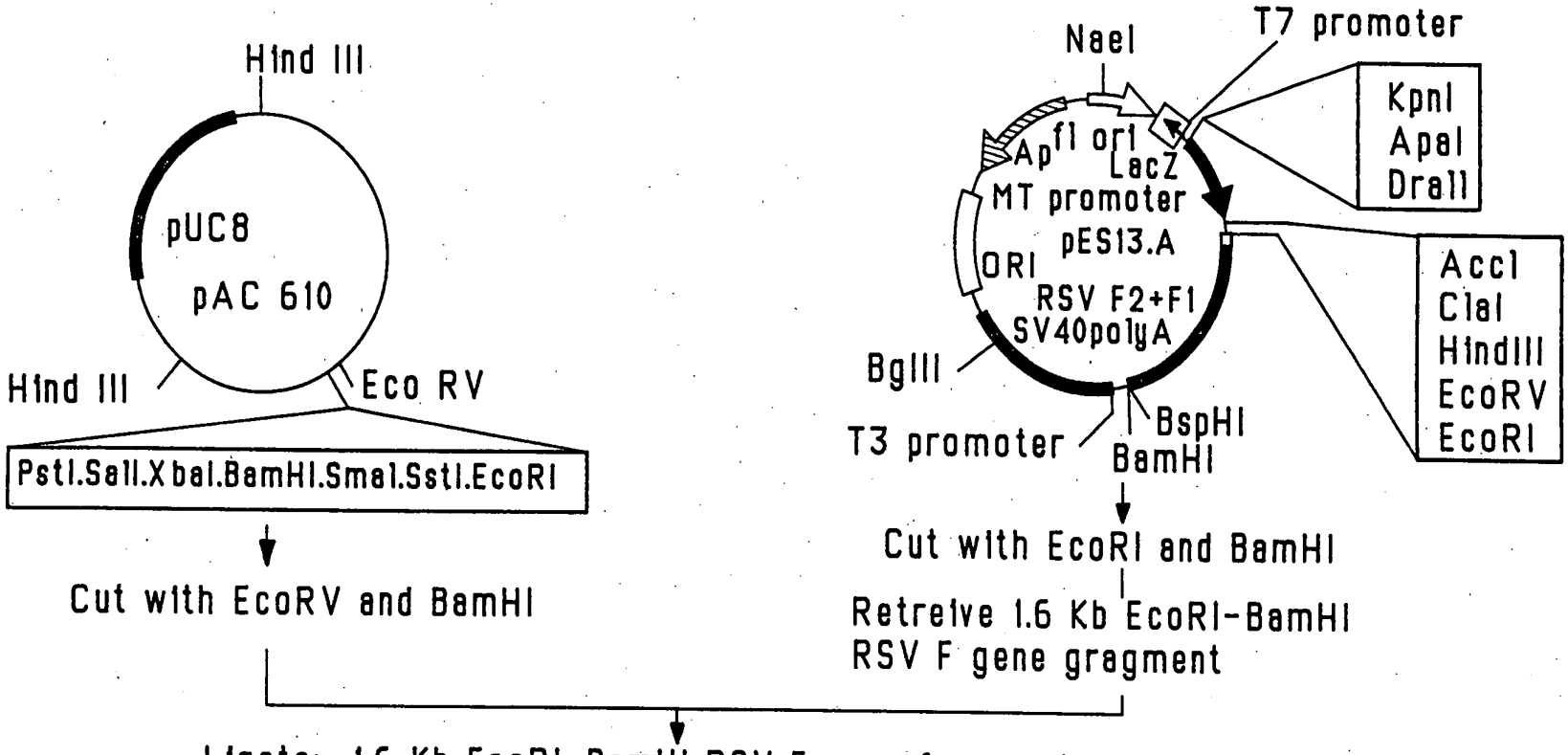


FIG.11.

FIG.12A. Construction of the modified pAc 610 baculovirus expression vector containing the chimeric F_{PIV-3}-F_{RSV} gene consisting of the PIV-3 F gene lacking both the 5' untranslated sequence as well as the transmembrane and cytoplasmic tail coding regions linked to the truncated RSV F1 gene



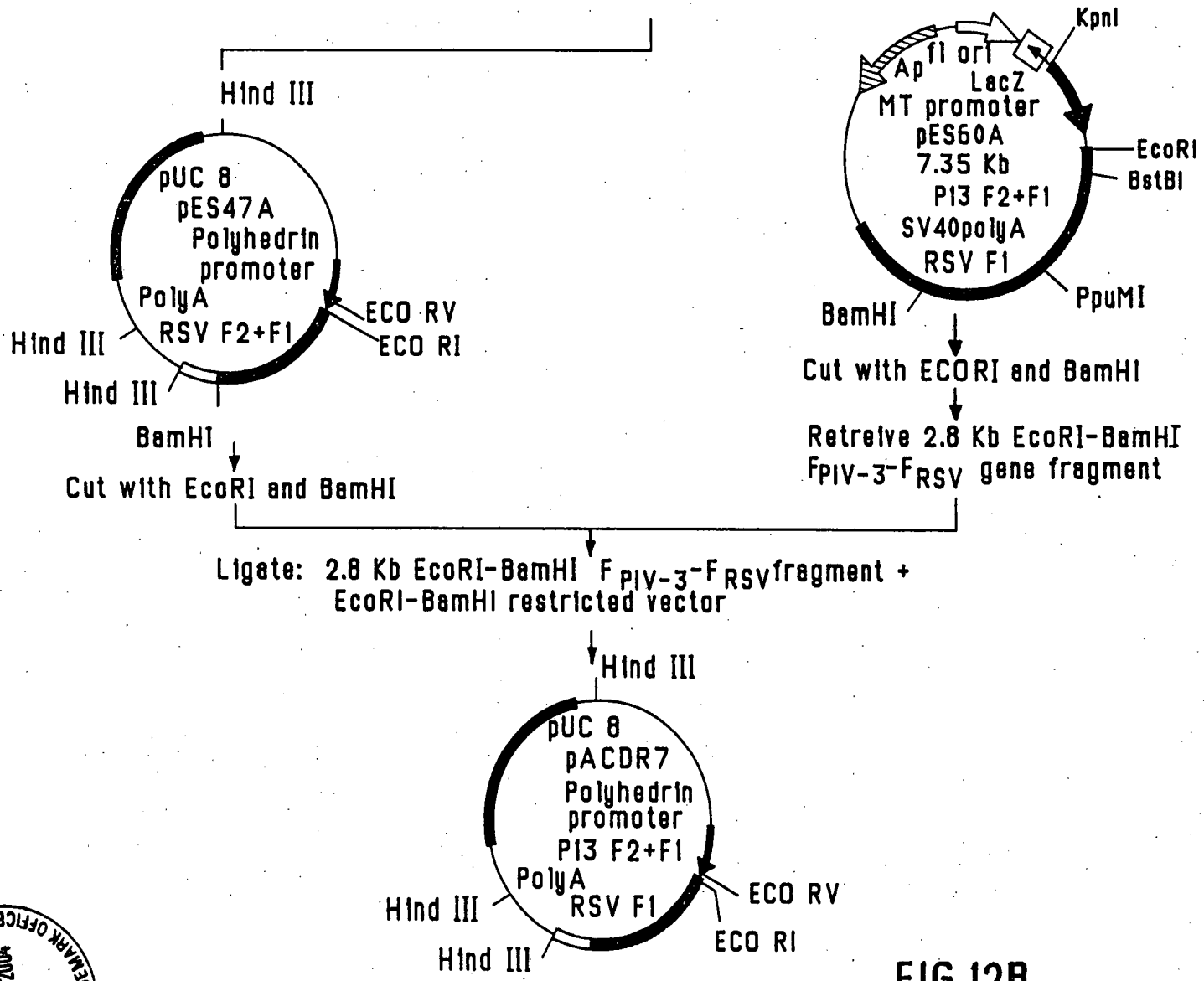


FIG.12B.

FIG.13

IMMUNOBLOTS OF CELL LYSATES FROM Sf9 CELLS INFECTED WITH RECOMBINANT BACULOVIRUSES

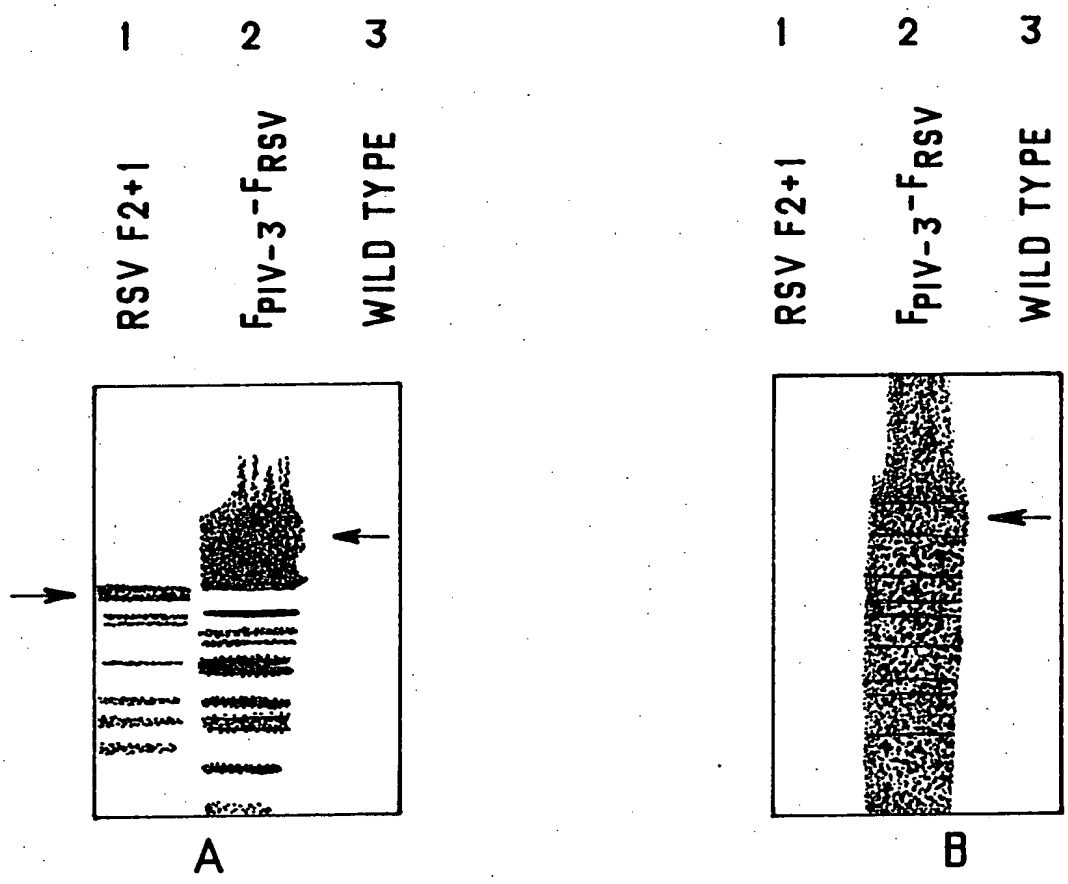


FIG 13 : Immunoblots of cell lysates from Sf9 cells infected wirth recombinant baculoviruses containing the truncated RSV F gene (Lane 1), the chimeric F_{PIV-3}-F_{RSV} gene (Lane 2) or infected with wild type virus (Lane 3) reacted with anti-F RSV Mab (panel A) and anti-F1 PIV-3 antiserum (panel B)

FIG.14. CONSTRUCTION OF THE BACULOVIRUS TRANSFER VECTOR pD2

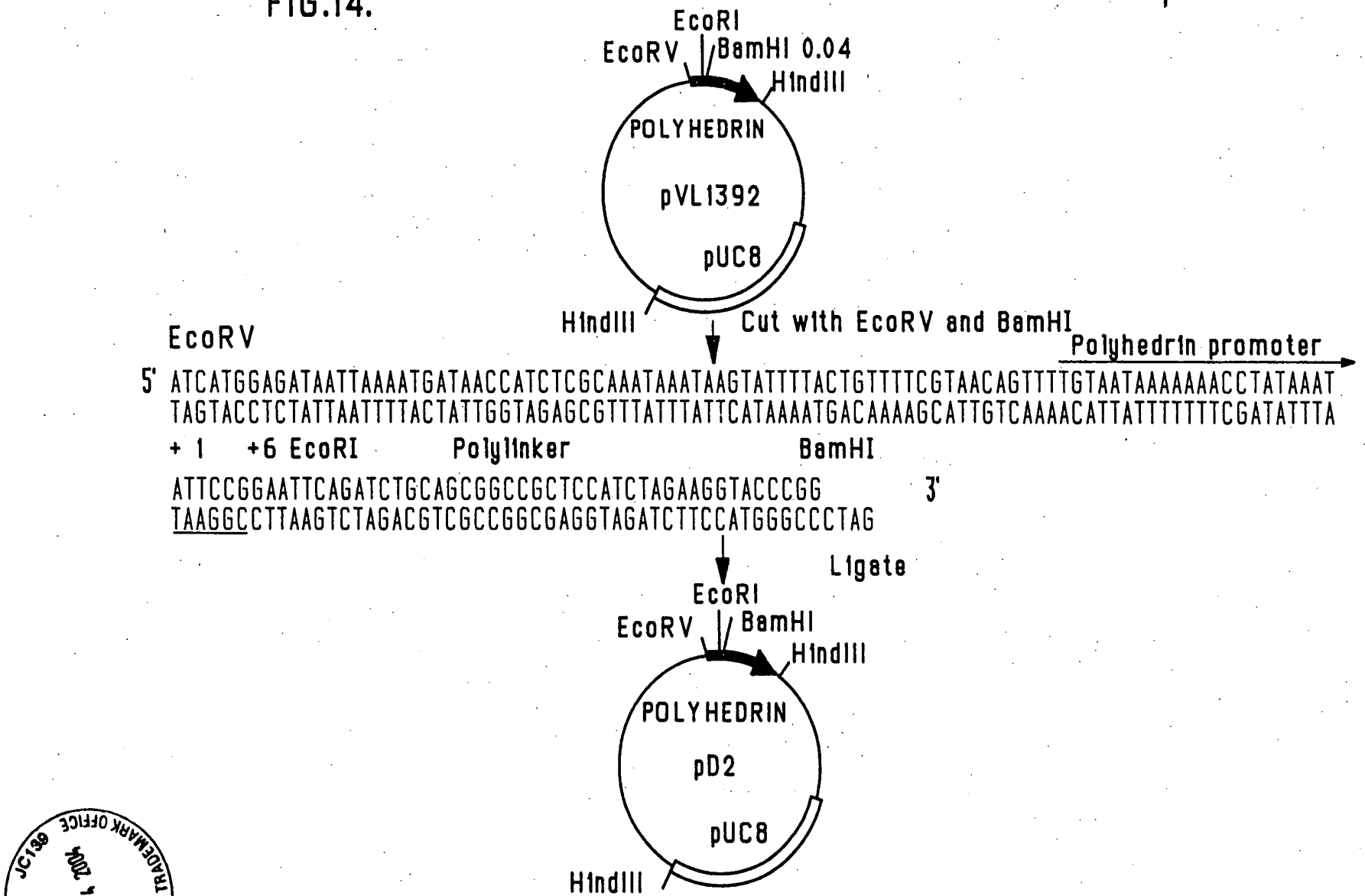


FIG.15A. CONSTRUCTION OF THE F_{RSV}-HN_{PIV3} CHIMERIC GENE

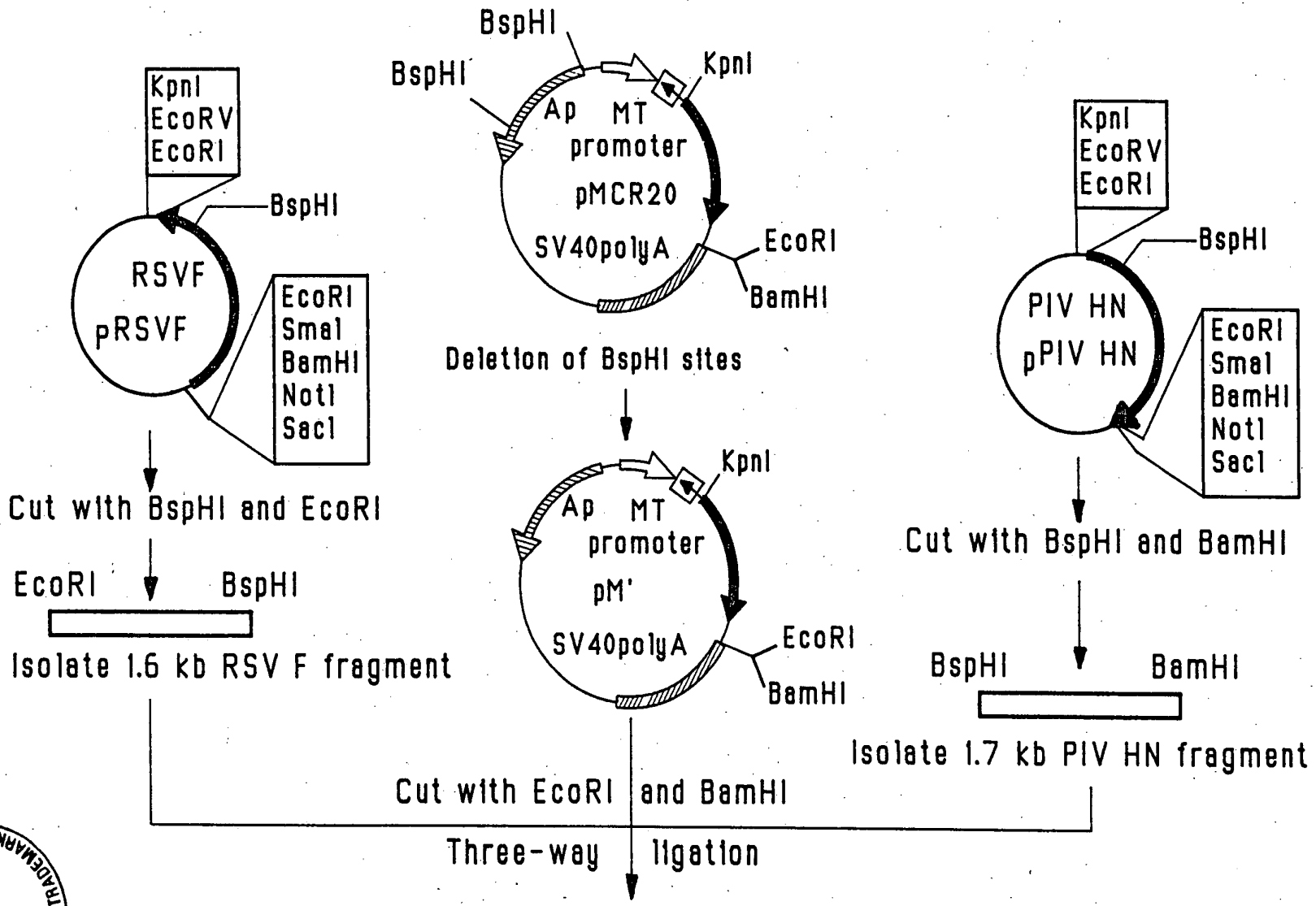


FIG.15B.

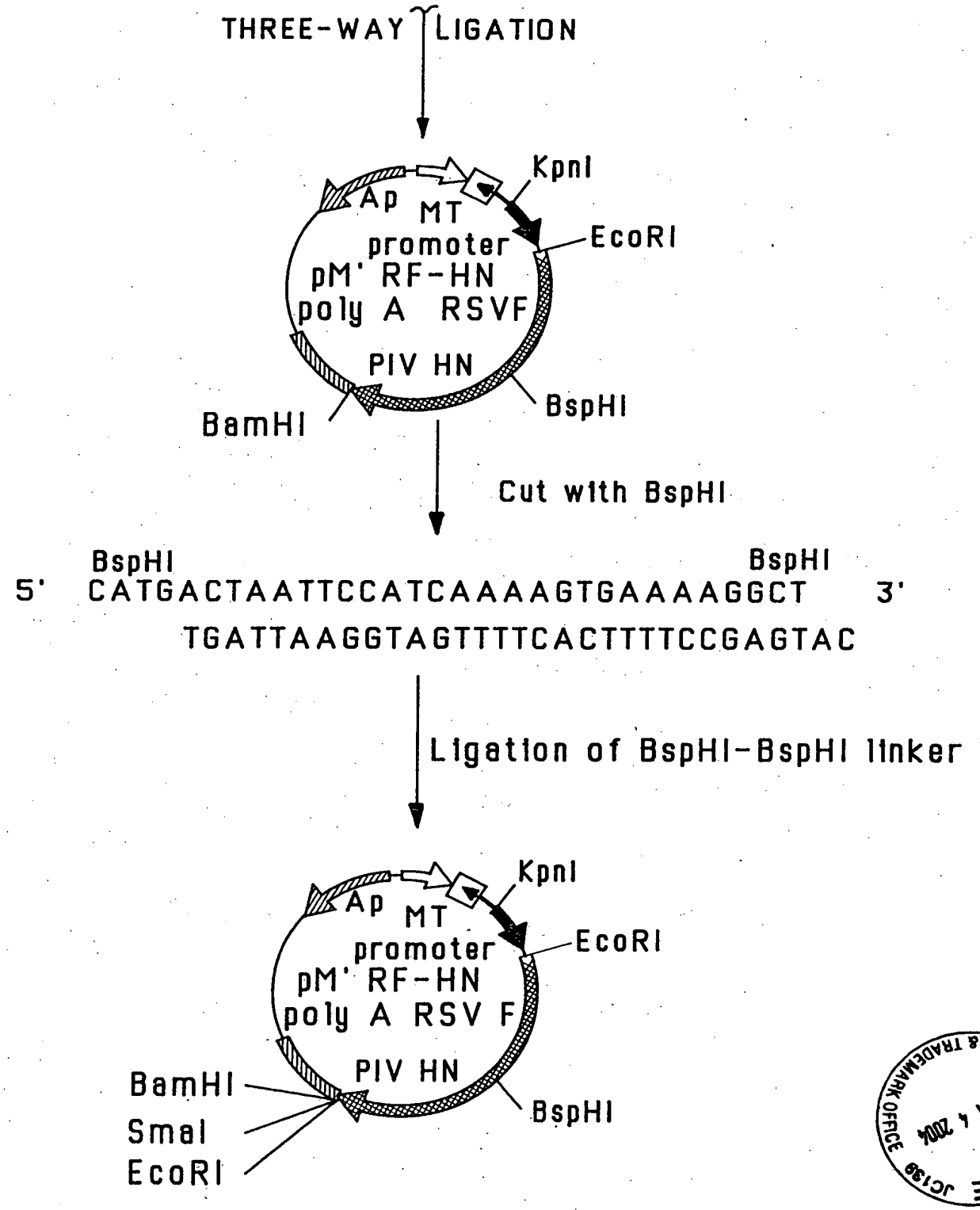


FIG.16

SDS POLY ACRYLAMIDE GEL AND IMMUNOBLOTS OF PURIFIED F_{RSV}-HN_{PIV-3} CHIMERIC PROTEIN

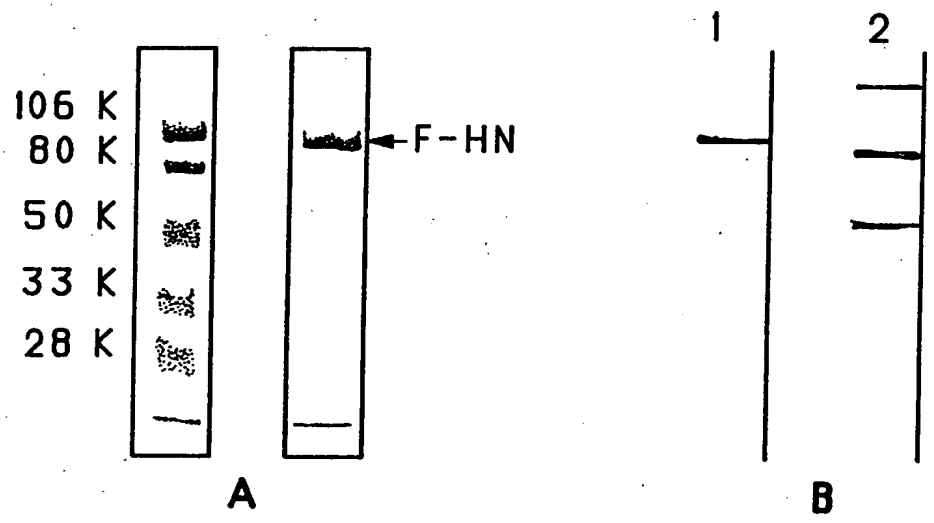


FIG 16 : A) Coomassie-stained SDS polyacrylamide gel of immunoaffinity- purified F_{RSV}-HN_{PIV-3}protein.

B) Immunoblots of F_{RSV}-HN_{PIV-3}protein reacted with an anti-F RSV Mab (lane 1) and anti-HN PIV-3 antiserum (lane 2)

FIG.17. MUTAGENESIS OF THE PIV-3 F GENE

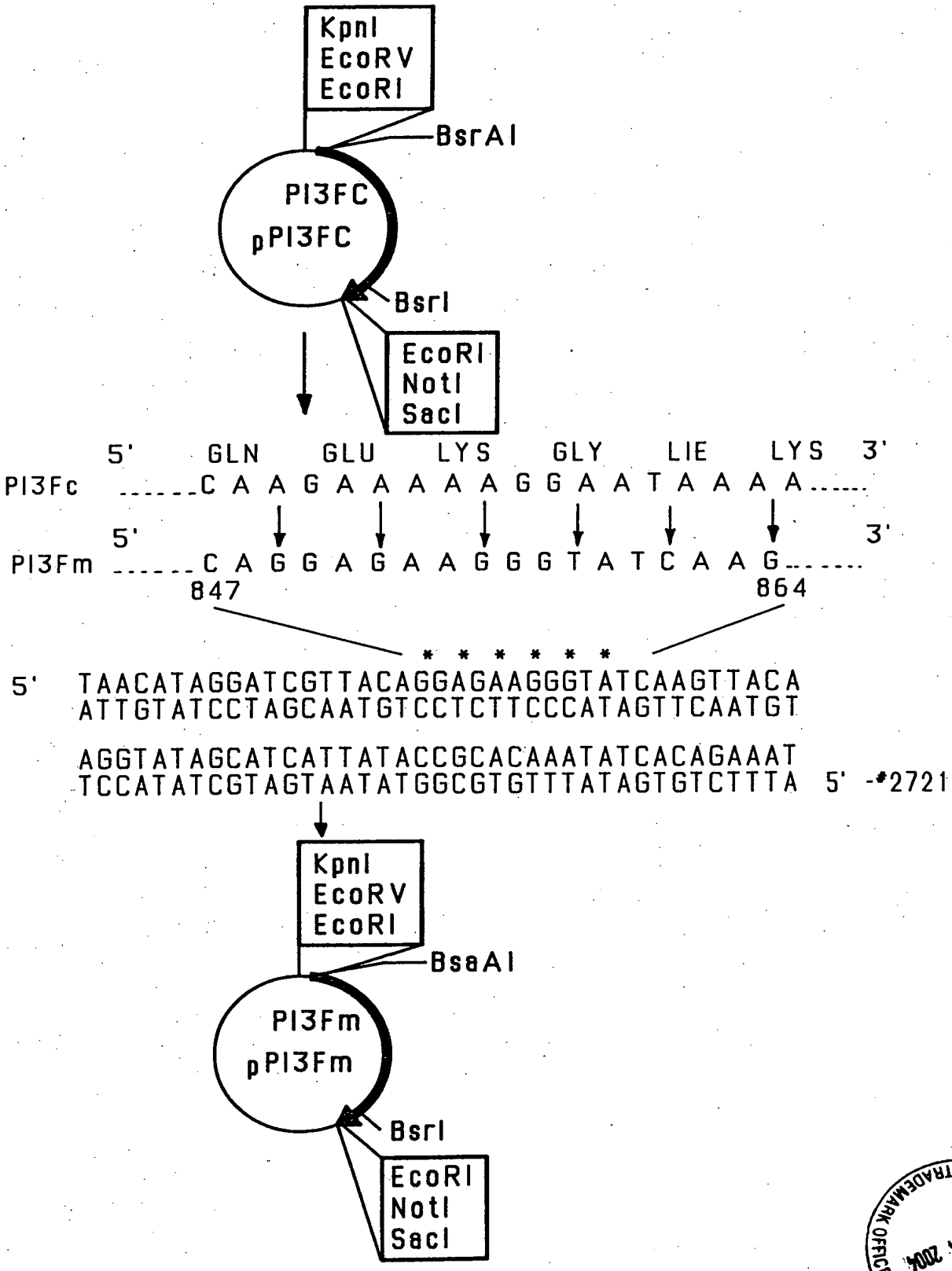


FIG.18. CONSTRUCTION OF THE F_{PIV3}-G_{RSV} CHIMERIC GENE